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GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS BEARING ON HEREDITY AND VARIATION

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GENETICS

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BEARING ON HEREDITY AND
VARIATION

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JOHN BELLING

JOHN BELLING, was born in Aldershot, England, October 7, 1866. He was educated in private and public schools, spending two years at the University of Birmingham and receiving the degree of B.Sc. at the University of London in 1894. After teaching in the lower schools he was research lecturer at the HORTICULTURAL COLLEGE AT SWANLEY, 1895–98, and for the succeeding three years, teacher of science at Wells, England, and Llandidloes, Wales.

In 1901 he was sent to the Island of St. Christopher in the West Indian service of the IMPERIAL DEPARTMENT OF AGRICULTURE. He remained in that service until 1907 when he visited Florida and joined the Staff of the STATE AGRICULTURAL EXPERIMENT STATION. After one year he became Assistant Botanist in the Experiment Station and Editor of the station publications and remained in this post eight years.

BELLING'S first scientific contribution dealt with the cytology of polyembryony in the mango, *Magnifera Indica*, published in 1908. Two years later he began his classical studies on the genetics of certain interspecific hybrids in the genus *Stizolobium*, which led to several discoveries of fundamental importance, particularly on the mode of inheritance of semisterility, which later found its cytogenetic explanation in his hypothesis of segmental interchange between chromosomes.

During the next five years BELLING was not officially connected with any institution but, in the latter part of this period, he became associated with DR. A. F. BLAKESLEE at the Station for Experimental Evolution of the CARNEGIE INSTITUTION OF WASHINGTON at Cold Spring Harbor, N. Y. From the first he devoted himself to the cytological aspects of the *Datura* investigations and in 1924 he was appointed Cytologist in the CARNEGIE INSTITUTION which position he held until his death FEBRUARY 28, 1933.

It was at Cold Spring Harbor that he began his intensive study of chromosomes and their behavior which was continued throughout the rest of his life and which entitle him to be known as pioneer in cell mechanics. For BELLING was the first, at least among botanists, to utilize both the genetic and the cytologic viewpoints in attempting to analyze the results of breeding experiments. He had unusual mathematical ability and a grasp of chemistry and physics which he applied in perfecting the technic necessary to success. His thorough understanding of optics is shown by his book on the microscope. His contributions to stain technology are of first rate importance. He developed the aceto-carmine method of staining by the addition of iron which made it possible to observe the chromosomes in stages of meiosis with greater facility and accuracy.

BELLING studied these phenomena in several plant species including *Canna, Cypripedium*, *Hemerocallis*, *Hyacinthus*, *Uvularia* and *Tulipa;* and in the *Datura* investigations which were one of his chief interests during this period he gave the final answer to many problems which the breeding behavior had raised and predicted genetic results from his observations of the chromosomes. His basal contribution, from which other hypotheses such as segmental interchange were developed, was his idea that the arrangement of chromosomes in pairs in the first meiotic division is due to the organization within the individual members of a pair such that like ends are attached in this stage. By identifying specific ends of chromosomes in groups of more than two he discovered evidence for the specificity of the extra chromosome in trisomics at a time when it was believed by some investigators that the extra chromosome is a characteristic rather than a cause of trisomic types and that it is a matter of no consequence which chromosome is extra. His influence in changing cytogenetic concepts can be further realized when it is remembered that until very recently it was held that the attachment of chromosomes into circles, so common in some species of *Oenothera* and so frequent in *Pisum* and *Zea*, is due to a general affinity between maternal and paternal chromosomes or that circle formation is a genetic character inherited like any other genetic character due to a gene.

As he had suffered from ill health for several years, in 1927 it was arranged that BELLING should go to California. At that time The Division of Plant Biology of the CARNEGIE INSTITUTION OF WASHINGTON AT STANFORD UNIVERSITY had not been established; so accommodations were provided by the Division of Genetics of the UNIVERSITY OF CALIFORNIA. At Berkeley he continued his work for the remainder of his life, holding the position of Research Associate in Genetics in the University without salary and continuing as Cytologist in the CARNEGIE INSTITUTION. Under these conditions he was able to renew his investigations with fresh vigor. Many different projects were planned and a surprising number were brought to completion within a comparatively short time. His first major undertaking was the study of the finer structure of the chromosomes. After much effort he was able to estimate the number of chromomeres in the pollen mother-cells of a lily and he showed that this number, about 2,200 pairs, agrees with expectations from genetic evidence, since the orientation and behavior of the chromomeres during cell division are such as would be expected if the chromomeres contain the genes. Then, by a special technic he demonstrated the existence of an ultra-microscopic particle near the center of each chromomere which he believed could be identified as the gene. Thus he anticipated, by his painstaking and critical research on normal chromosomes in plants, the important discoveries of structure and organization of chromosomes which have come recently from investigations on the salivary glands of certain insects. It was in connection with these studies that he perfected the iron-brazilin method of staining which he found more satisfactory for some materials and for certain purposes than the iron-aceto-carmine method.

During the last three years of his life, BELLING concentrated his efforts with unabated enthusiasm and increasing clarity of penetration on a problem of major importance to genetics, viz., the nature of the mechanism of crossing over or interchange of segments between chromosomes. Two days before his death in 1933 he brought to completion the manuscript of his final paper in a series of valuable contributions on this complex problem. This paper (*Genetics*, 18: 388–413, 1933) presents the evidence for his hypothesis and its application to cogent genetic data. In order to convey more clearly his conception of the mechanism of crossing over and other chromosomal rearrangements, he prepared a series of models which were exhibited by the Carnegie Institution in Washington and New Orleans in 1931–32 and some of them were shown at the Sixth International Congress of Genetics in 1932.

It was only by combining unusual skill in technic and observation, rare ability in formulating hypotheses with a strong factual foundation, and a dogged determination to succeed in spite of all obstacles, including his own physical frailty, that John Belling was able to win the place of honor he deserves in the early development of the new science of cytogenetics. Always timid and retiring he preferred to work in obscurity, and indeed the only public recognition he received was the honorary degree of Sc.D., awarded by the University of Maine in 1922. Yet to a few who came to know him intimately, either by personal contact or through his published poems, Belling revealed an unsuspected philosophical insight and a truly artistic temperament. Rather late in life he married DR. HANNAH SEWALL, a woman of culture and refinement. The short span, some five or six years, before her death seems to have been a period of great spiritual uplift for him. Some of his poems, particularly the "Elegy," reveal how deeply the artistic side of his nature was influenced by this all too brief companionship.

In his own laboratory BELLING was always at home and ready for visitors. To students especially he gave a cordial welcome and a sympathetic hearing. Impatient of mere conjecture without factual basis, he was extremely careful in his own writing and he set a standard of excellence in this, as in all his work, which may well serve as a guide and inspiration for others.

This biographical sketch was prepared by Professor E. B. Babcock in collaboration with Dr. A. F. Blakeslee who a so furnished the photograph for the frontispiece.

GENETICS

1916-1936

WITH this issue GENETICS enters its twenty-first year. From its inception it has been conducted by the Editorial Board of ten members which founded it, and by two editors who have served it for periods of ten years each. To the first editor Professor George H. Shull and to the retiring editor Doctor Donald F. Jones, the Board and all students of Genetics owe a great debt of gratitude for their effective and unselfish services.

GENETICS is owned by its Editorial Board, a self perpetuating body which participates actively in publication and management of the Journal. The original board has recently enlarged itself by the election of five new members, Leon J. Cole, John W. Gowen, Alfred H. Sturtevant, Sewall Wright, and L. C. Dunn. The last named assumes the editorship with this issue. On the occasion of its twenty-first birthday GENETICS welcomes the new members to its Editorial Board.

INHERITANCE AS IT AFFECTS SURVIVAL OF RATS FED A DIET DEFICIENT IN VITAMIN D

JOHN W. GOWEN
Rockefeller Institute for Medical Research, Princeton, New Jersey

Received August 17, 1935

THOSE familiar with experiments on diets realize that within a group of animals given the same food in like quantity, the reactions of individuals may be quite diverse. On another diet a similar group may show similar individuality but the curve of such variation may be distinctly separate from that of the first group. Experimentation in nutrition has attempted to eliminate individual variation in the animals and to emphasize the differences between given diets. In the light of the objectives sought, this attitude is commendable. Individual variation within experiments, however, seems to have a significance not to be overlooked. Individuality as a cause of variability has the same primary importance with respect to the characteristic pathology which animals develop under experimental dietary conditions as under those of nature. The food requirements of even closely related genera may often be quite distinct. From the point of view of evolution, this seems significant since the causes of such differences in food requirements are fundamental to the correlation between the spread of animals and their subsequent development.

For the study of diverse individual variations which animals may show toward a given diet, vitamin D was chosen as the major variable in our experiments. This nutrient principle is apparently a single chemical entity necessary for normal body development and maintenance of many vertebrates. The symptoms which develop in the absence of it depend somewhat upon the age of the animal. A lack of it in the young, when bones are developing actively, may lead to rickets and osteoporosis, due to improper deposition or actual extraction of calcium from growing skeletal structures. Growth is impaired or stopped; teeth are poorly calcified and often pitted. A deficiency at puberty may be correlated with crippling and painful changes which often lead to difficulties in reproduction. Such conditions are more striking in females than in males. In the pregnant mother, excessive fetal demands for calcium and phosphorus may induce symptoms of osteomalacia. Later, the nursing mother often shows somewhat the same pathological conditions due to similar demands in the production of milk. In middle-life, osteomalacia sometimes develops if the diet is sufficiently inferior, although the changes tend to appear less rapidly than in the young. Birds show particularly severe symptoms in the form of leg-weakness which may be rapidly fatal in the young chick.

Tetany often follows long-continued deprivation in some cases. Deficiency is also accompanied by reduction and degeneration of the spleen, deposition of calcium in the walls of arteries, a reduction of blood-clotting time, hemorrhage and striking change in the parathyroids. An increase in the size and number of epithelial cells of the parathyroids is followed by shrinkage and regression of the cell cords and, in some cases, a hyperplasia of the stroma. Marked keratinization then takes place. The antirachitic principle may be supplied through food or through exposure of the body to direct sunlight. In the latter case, the color of the exposed skin materially influences the amount of sunlight absorbed and hence its antirachitic effect (1).

The question which will be studied in this paper is the effect of inheritance on the utilization of vitamin D. The criterion for measuring the effect is the variation in the length of time that the animals survive when fed a diet deficient in the vitamin.

MATERIAL

Genetic research has shown that a population of animals bred at random, or nearly so, generally contains different inherited potentialities. Two individuals, though they be carefully selected are apt to deviate in their reaction to any given variable even if the experiment is well controlled. It is evident that this random differentiation would vitiate the analysis we propose. The aim is to keep the full spread of inherited differences of the initial population, but to segregate it into lines each of which has a distinctly reduced inherited variation. Close inbreeding of the brother and sister type furnishes a means by which this end may be accomplished. If from a population of rats, we select a dozen pairs, we have a sample of the inherited potentialities of the entire group. Any given pair will be likely to contain but a limited part of the variation in the total spread of inheritance. The progeny of such a pair will tend to have only a portion of the inherited variation which their parents possessed, although considered collectively, the whole litter, if large, might well contain all of it. Each generation, so long as the inheritance of the line comes through a single pair, will consequently become more and more alike, until a time is reached when the progeny within a given generation are so closely similar that they can be considered essentially alike in respect to inheritance. Any variation which such a population shows may therefore be attributed to environment. Those familiar with the effects of such factors as linkage and balanced lethals will realize that even here difficulties may enter. However, the same procedure in inbreeding each of the sample lines originally selected at random will result in uniformity within each of the lines, but between the lines, if the selection is fortunate, the full inherited variation of the original random population will remain. The differences between such lines may be used to measure the influence which inherited variation may exert on the particular variable studied.

The experiment described here was fortunate in having available to it 16 lines so distinctly differentiated in their inheritance. We are indebted to Dr. M. R. CURTIS of the INSTITUTE FOR CANCER RESEARCH at COLUMBIA UNIVERSITY, New York, for the animals which we used to start each of our lines, and for the data from which it has been possible to obtain the survivorship of these lines when fed our complete diet (CURTIS and BULLOCK 1923; CURTIS, DUNNING and BULLOCK 1933). The sixteen strains were originally from the stock rats of four different dealers and from one European laboratory strain. When the selected pregnant females were transferred to Rockefeller Institute at Princeton, New Jersey, the different lines had been bred brother by sister for 6 to 9 generations. Three to 5 generations of further inbreeding intervened before the animals were used in the experiment. Care was taken to avoid the introduction of transmissible diseases and parasites into these new lines. Before transfer, the pregnant females were examined thoroughly and dipped to eliminate any external parasites. After transfer, they were separated from each other by solitary isolation in different laboratories. Here the young were born and at weaning time the mothers were autopsied. Lungs were examined especially for pneumonia, feces for paratyphoid bacilli and the eggs of nematode worms. All females abnormal in any of these particulars were eliminated along with their litters. The remaining litters were then bred and the process repeated in like manner. Finally the stock was free of paratyphoids, nematodes, skin parasites and pneumonia, for a time at least. Unfortunately we missed the middle ear focus of the actinoides organism and as a result the pneumonia which it favored subsequently appeared in our stock. We could not find that its incidence had any distinct relation to the deaths in different lines whether they were subjected to the vitamin D deficient diet or to the normal one. That the disease is capable of control by the measures taken is indicated by the fact that it has been eliminated in one strain of our stock (NELSON and GOWEN 1930).

The untreated rats were maintained on the following stock ration. Bread, whole milk, fresh vegetable and yellow corn were fed on Monday, Wednesday and Friday; bread, milk and cereal on Tuesday and Thursday; bread, milk, cereal and yellow corn on Saturday. The cereal was composed of equal parts of cracked whole wheat, rolled oats and yellow cornmeal mixed with milk and cooked for 20 minutes. A special rat biscuit and water were before the animals at all times. Meat was fed once a week. Such a diet was chosen for the untreated rats because of its wide variety of foodstuffs and its ability to maintain the animals' condition.

The choice of a diet for the test animals presented many difficulties. Two diets low in vitamin D were in common use; that of McCollum, 3143 (1922), and that of Steenbock and Black, 2965 (1925). Both rather readily produce the vitamin D deficient picture in rats fed on them. The calcium-phosphorus ratio is unbalanced in both, the calcium being high and the phosphorus low. Furthermore, both diets are believed to be deficient in vitamin B_2, and also in certain minerals and iodin. Either diet, however, has in its favor the real advantage of having been widely used in other feeding experiments. Since comparability with the experiments of others seemed desirable, it was decided to use the 2965 diet in preference to any new ration which we might devise. The proportions of this diet were 76 percent of ground yellow corn especially selected for its brightness of color, 20 percent of wheat gluten, 1 percent of sodium chloride and 3 percent of calcium carbonate. Distilled water was continuously before the animals.

The experiment was conducted in the following manner. Rats to be fed the vitamin D deficient diet were kept out of contact with direct sunlight. Females were isolated from the time that pregnancy was noted and the resulting litters were kept separate from other rats. The litters were weaned 3 weeks after birth and from then until 46 days of age, when the vitamin D deficient diet was started, they were fed on the regular stock ration. At 46 days of age, the young were put into individual screen-bottom cages 9 inches square and held $\frac{3}{4}$ of an inch above the shelf and away from direct sunlight. Food was always present in the cages. Its daily consumption was reckoned on the difference between the weight put into the cage on one day and that remaining the next. It is evident that animals could obtain some of the metal elements from the wire of their cages; other than that and their diet, it is believed that they received nothing. In the daily routine procedure, all cages were examined for feces which might have caught on the wire bottom, and shelves under the cages were cleaned. The animals were weighed once each week.

Data pertinent to the problem included litter size, initial weight at 46 days of age, maximum weight, age when maximum weight was attained, time of death and weight at death. From these items the rate of gain and absolute gain from initial to maximum weight may be calculated. The subsequent loss in weight and rate of loss until death may also be determined. The food intake was found to rise somewhat at first, then to remain quite constant until the week of death when the records show some drop. In view of the fact that differences in litter size and in weight are regarded as in part hereditable and since the aim of the experiment was to test inheritance, no adjustment in litter size and weight was made. Obviously such a procedure would have overthrown some of the objectives of the experiment. This problem is approached, however, by what is possibly a better technique in a later section of the paper.

Originally it was intended to revive some of the long-time survivors of the diet deficient in vitamin D by giving them a normal diet and ultraviolet light. The plan was not carried far because rats, although revived in many respects by such treatment, were found to be sterile.

It is clear that although the experiment is directed at the effect of inheritance on a deficient supply of vitamin D, other more or less significant nutrients have been reduced. We should, of course, like to say that the results obtained relate entirely to vitamin D and the effects of inheritance in making this element more or less essential for the metabolism of one strain but not indispensable to a strain of different genetic constitution. This, it seems, we can not do completely, if for no reason other than our present ignorance of many essentials of nutrition. All the experiment can hope to indicate is that given a diet with certain deficiencies, vitamin D important among them, the susceptibility of different groups varied, and that a portion of this variation was attributable to inheritance. In referring to the subsequent results the term "vitamin D deficient diet" is intended to convey the broader definition. Considered from the point of view of evolution, such variations may indicate the adaptability of certain races to border zones deficient in particular environmental factors and in DAVENPORT's sense may fit races for invasion into what would otherwise be inhospitable regions.

The present paper directs attention to the variation in length of life of genetically different strains of rats fed the same vitamin D deficient diet. We may begin our study, however, by an analysis of the variation curves of the lives of all of the rats taken together. Through the courtesy of Dr. M. R. CURTIS in supplying the data, it has also been possible, for the benefit of the reader, to place on the charts life curves of rats of the same strains when fed a completely adequate diet. A total of 4981 male and 7607 female completed lives are represented in this material.

LIFE CURVES OF RATS ON THE VITAMIN D DEFICIENT DIET

Data from all lines were combined into a life curve representing the effects of the vitamin D deficient diet on the whole population. The total data compared with those for the human life tables are rather pitifully small, for collecting material under the conditions and requirements of the experiment is slow work. But surprisingly smooth curves result. There are also the real advantages that the data were collected with a definite purpose in view and that all the entering variables were recorded at the time of occurrence and not at death.

SEX DIFFERENCES IN THE REACTION TO THE VITAMIN D DEFICIENT DIET

Sex, of course, is an inherited character, being dependent on the chromosome distribution, although the conditions bringing it about have thus far defied exact analysis. Since a sex difference is manifest in the data, the original curve is divided into 2 curves, 1 for each sex. The tabulation represents survivors against duration of life, duration of life being measured as age in days divided by 30. The males in table 1 show a somewhat greater duration of life than the females.

TABLE 1
Survivorship curves of rats on vitamin D deficient diets.

AGE DAYS/30	NUMBERS WHICH SURVIVED	
	MALES	FEMALES
2	170	167
3	162	154
4	141	139
5	108	104
6	78	73
7	60	50
8	44	29
9	32	15
10	25	8
11	16	2
12	11	1
13	6	
14	5	
15	4	
16	1	

Figure 1 gives the graph of these results. If we test the significance of differences between the sexes by comparing the specific death rates, we find that the χ^2 value for 10 classes is equal to 14.7; the P value is slightly less than 0.1 (ages of 10 and above are grouped into one class). We should have to conclude that sufficient data were not at hand to prove that there was a distinct difference between the sexes. But the fact that a sex difference is manifest in our data in the direction of a more severe effect of the vitamin D deficiency on females than males is in line with observation on the human in puberty and after-life and in my own unpublished results on the domestic fowl. A similar effect has been noted by BILLS and others (1931) in line test studies on rats, the males showing a slightly greater rate of healing than the females.

Sex differences in reactions to specific agents of disease and to other agents are becoming rather well known. WRIGHT and LEWIS (1921) have shown such differences in the resistance of guinea pigs to tuberculosis. In their experiments, as in ours on vitamin deficiency, the females died more rapidly than the males. In mice the writer's experiments are showing a reversal of this effect: exposure of a population to the effects of ricin poison causes the males to die at distinctly greater rates than the females (GOWEN and SCHOTT 1933). In rat and mouse typhoid, and mouse liver disease (*B piliformis*), however, the sexes react alike (IRWIN 1929; SCHOTT 1932; WEBSTER 1933; GOWEN and SCHOTT 1933).

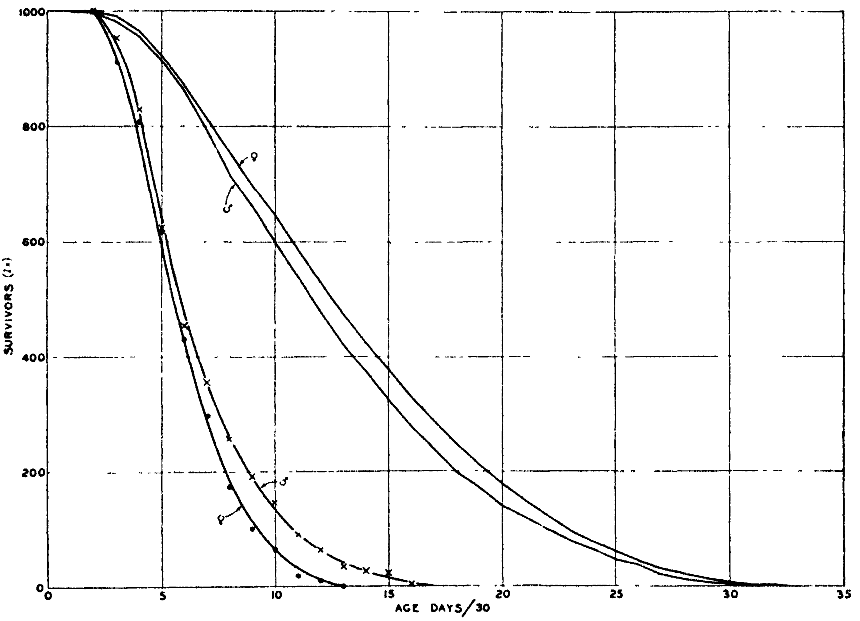

FIGURE 1.—Curves showing the survivorship plotted against age for rats on vitamin D deficient diets, left-hand curves, and like rats on a normal diet (based on CURTIS' data), right-hand curves.

A comparison of the left- and right-hand curves in figure 1 shows that rats on a diet believed to be adequate have a distinctly longer life-span than those on the deficient diet. In the former group, 11–12 months elapses before 50 percent of the population dies, in the latter only 5–6 months intervenes. After this point, the survivors of the deficient diet begin to die off more sharply than the others and the form of the curve therefore changes. This type of curve is also found when Drosophila are subjected to starvation. Apparently when one major cause of death overshadows all others the curves tend to have an abrupt, relatively limited cycle. This suggests the destruction of a reserve substance at a given rate. The fact that the rats on the vitamin D deficient diet lived but one half as long as those on the complete diet is sufficient evidence to emphasize the importance to life of the deficiencies observed in this diet. These differences are without regard to the inheritance since the inheritance within two groups is quite similar.

The sex differences in rats on the normal diet are well marked, no question of their statistical significance being possible. They are, however, in a direction opposite that of rats on the vitamin D deficient diet, that is, the males die sooner on the normal diet than the females. Such a difference between the rats on the two diets seems significant, for while it is not possible to assert that the male and female rats are strictly not identical, the probability that the females would live longer than the males, as they do under the normal diet, becomes rather small, about 1 in 100.

CHARACTERISTICS OF THE CURVE SHOWING THE FREQUENCY OF DEATH

The constants of the frequency curves of the population, variable in its heredity, have special interest when they are compared with those of the like population when the variation brought about by the heterogeneous heredity is removed. The frequencies of death against age are shown in table 2 for the population lacking vitamin D.

TABLE 2

Frequency of deaths of rats on a vitamin D deficient diet.

AGE DAYS/30	NUMBERS WHICH DIE	
	MALES	FEMALES
2	8	13
3	21	15
4	33	35
5	30	31
6	18	23
7	16	21
8	12	14
9	7	7
10	9	6
11	5	1
12	5	1
13	1	
14	1	
15	3	
16	1	

Figure 2 shows the frequency polygons, in which percentages of death are plotted against age for rats on normal and vitamin D deficient diets. The two sexes are represented separately. The striking feature of these diagrams is that animals on the vitamin D deficient diet reach a much higher total death rate per unit of time than those on the normal diet. For purposes of comparison, the constants of PEARSON'S frequency curves are useful in clarifying these differences. These constants are found in table 3.

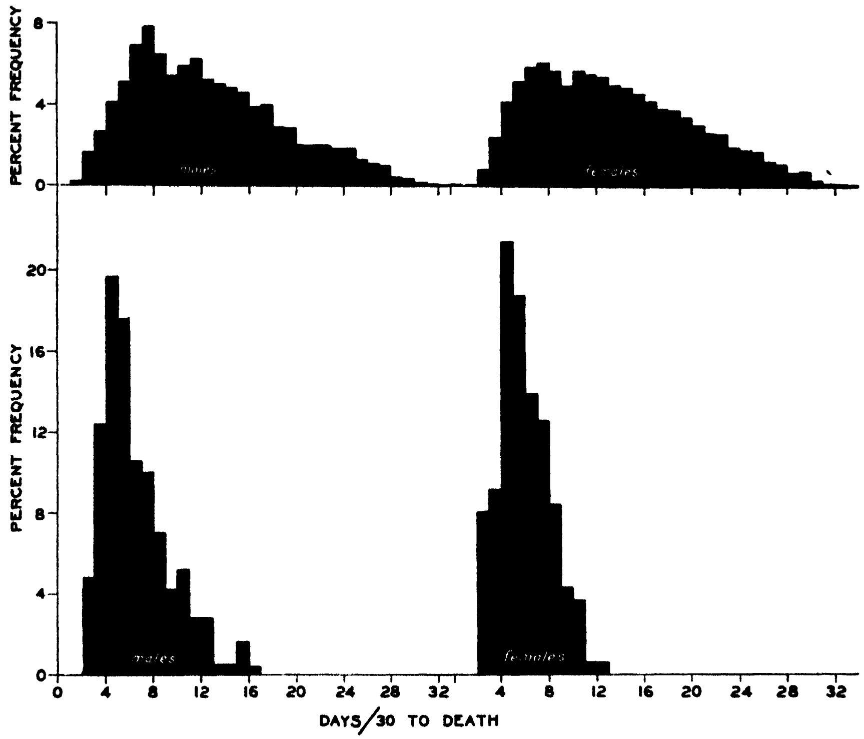

FIGURE 2.—Frequency diagrams for percentages (upper figure) of death plotted against age for rats on normal diets (derived from CURTIS' data), and on vitamin D deficient diets (lower figure).

TABLE 3

Frequency constants for deaths of rats plotted against age, normal and vitamin D deficient diets

	VITAMIN D DEFICIENT DIET		NORMAL DIET (CURTIS DATA)	
	MALES	FEMALES	MALES	FEMALES
Mean (days)	197.3 ±4.6	178.3 ±3.3	379.4±1.8	403.6± 1.5
Standard deviation	89.0 ±3.3	63.4 ±2.3	189.9±1.3	198.1± 1.1
Coefficient of variation	45.2	35.6	50.1	49.0
μ_2	8.80	4.46	40.13	43.6
μ_3	28.85	4.77	159.40	151.3
μ_4	299.22	56.13	4406.85	4840.4
β_1	1.22± .27	0.26± .11	0.39± .02	0.28± .01
β_2	3.86± .48	2.82± .25	2.74± .04	2.55± .02
Skewness	1.27± .38	0.41± .10	0.77± .04	0.70± .03
Type of curve	I	I	I	I

The constants of table 3 show that the frequency distribution of the deaths of rats on the vitamin D deficient diet has a distinct positive skewness. The curve prescribed for these distributions is PEARSON's Type I.

If we compare the constants derived for animals on the vitamin D deficient diet with those for a similar group on the normal diet (CURTIS' data), we note that while the average duration of life in the latter animals is not quite twice as great as that of the former, the standard deviation is more than twice as large. The coefficient of variation consequently shows a relatively greater variation in the deaths of rats on the normal than on the vitamin D deficient diet. The skewness of both curves is within the same range. The constants of both sets of observations lead to Type I curves.

CHARACTERISTICS OF THE FREQUENCY DISTRIBUTIONS OF DEATHS OF THE SEXES WHEN EACH GENETICALLY SEPARATE GROUP IS CENTERED ON ITS MEAN

We may turn to the question, what are the characteristics of the death curves when the hereditable variation in the susceptibility of the animals is removed? Inbreeding tends to purify a race in the sense of making one animal genetically like the other. The strains of rats used in these experiments had all been inbred nine or more generations by brother and sister matings. They should, therefore, be quite pure from the point of view of inheritance. The variation which remains within each strain may be looked upon as that due to external environmental conditions. Any differences between the strains, on the other hand, may be assigned to differences in the genetic constitution of the strains. By centering the variability curve of each strain on its mean, we eliminate the variation due to heredity and are in a position to examine the variation due to environment. The constants of the distributions of deaths for male and female rats on the vitamin D deficient diet are seen in table 4.

TABLE 4

Constants of the distributions of deaths for rats on vitamin D deficient diet when each genetically separate group is centered on its mean.

	MALES		FEMALES	
Mean	−0.0	days	−0.2	days
Standard deviation	56.1 ± 2.0	days	42.16 ± .6	days
μ_2	3.49	days	2.02	days
μ_3	1.98	days	0.36	days
μ_4	52.85	days	13.39	days
β_1	0.092 ± .186	days	0.015 ± .010	days
β_2	4.187 ± 2.43	days	3.036 ± .365	days
Skewness %	0.096 ± .083	days	0.061 ± .064	days

When each genetically separate group is centered on its mean, the standard deviations have become markedly reduced. This shows that the strain differences played a real part in the resistance of the population to the lack of vitamin D. The frequency distributions have become more symmetrical, the Beta constants do not differ significantly from the values of the normal curve $\beta_1 = 0$ $\beta_2 = 3$. The skewness of both the male and female groups has been eliminated with the removal of the major inheritance differences. The frequency curves are now essentially symmetrical.

The constants indicate that when the heterogeneity introduced by differences in inheritance is removed, Gaussian curves will approximate the survival curve of the rats. This fact has particular significance to studies of the potencies and effects of vitamin D preparations since the interpretation to be placed on such assays so often depends on the statistical significance to be attached to them. The approximation of these distributions to normal curves has a further interest to other studies of physiological resistance, that is, the effect of inheritance on disease resistance, since it is often necessary to determine the perimeters of the curves for the resistance of the host to the given disease-provoking entity before an analysis of the inheritance of disease resistance is possible.

EVIDENCE FOR THE EFFECTS OF INHERITANCE ON RESISTANCE TO VITAMIN D DEFICIENCIES

The mean durations of life of the different lines of rats, together with their standard deviations are given in table 5.

TABLE 5

Means and standard deviations of the durations of life of the different inbred lines of rats.

LINE	MEAN		STANDARD DEVIATION	
	MALES	FEMALES	MALES	FEMALES
1	233 ± 9	210 ± 7	54 ± 6	56 ± 5
6	289 ± 18	258 ± 10	90 ± 13	37 ± 7
7	133 ± 7	164 ± 11	34 ± 5	55 ± 8
8	295 ± 14	221 ± 9	82 ± 10	46 ± 7
10	217 ± 11	227 ± 11	54 ± 8	50 ± 5
11	132 ± 6	132 ± 5	33 ± 4	35 ± 4
12	160 ± 5	158 ± 6	21 ± 3	40 ± 4
13	344 ± 15	274 ± 9	78 ± 10	49 ± 6
14	160 ± 7	151 ± 5	45 ± 5	36 ± 4
15	144 ± 6	155 ± 9	43 ± 4	44 ± 6
16	143 ± 7	116 ± 6	45 ± 5	40 ± 4

The data show several significant differences in the average duration of life between the lines. Such differences may be appreciated best by comparing the bar diagrams in figure 3. The height of the bars represents the average duration of life of the line indicated by number at the base of the bar. The left-hand portion of the bar represents the males; the right-hand portion the females. Evidently lines 6, 8 and 13 survived for the longest period on the vitamin D deficient diet. Lines 1 and 10 lived a shorter time, while the remaining lines showed the poorest survival.

The data show that the two sexes within a given line correspond fairly well in their duration of life. It is likewise evident that the lines which tend to live the longest time are those which show the differences between the sexes.

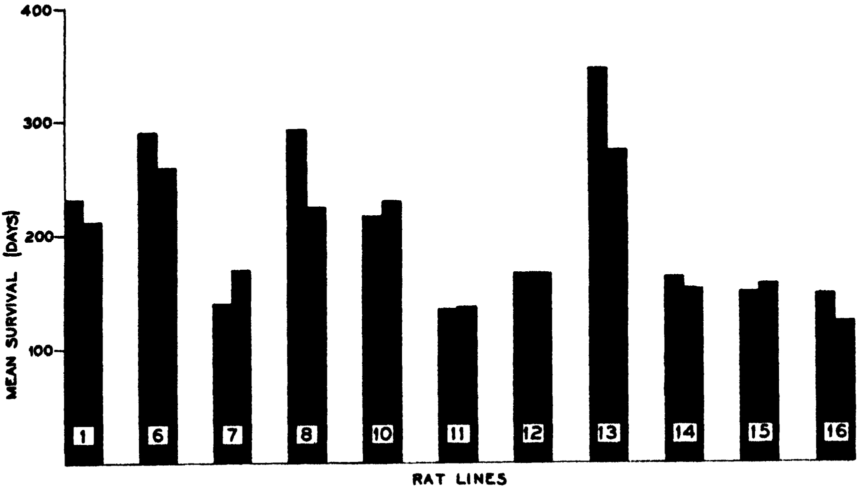

FIGURE 3.—Average duration of life of genetically differentiated lines of rats fed on a diet deficient in vitamin D. Left-hand side of bars, males, right-hand side, females.

The relative effect of inheritance may be more clearly demonstrated by a rather precise comparison. The central idea behind inheritance is that closely related individuals, on the average, will resemble each other more than will unrelated ones. The degree of resemblance between any two relatives may thus be determined as the quantitative difference between the measurements of a particular character in the two individuals. Genetically unrelated animals composing another group may be treated in a similar manner. The difference between measurements for the related and unrelated or random sample groups will give a quantitative value for the effect of the inheritance. If now, we desire a direct measurement of the amount of genetic correlation between the related individuals in the groups the summed squares of these differences will give such a measure of this inheritance effect.

The total squared differences for the durations of life of the whole population of rats on this vitamin D deficient diet may be divided into two parts; the variation due to differences between the different genetic lines and the variation due to the random variation of the individuals within the lines. Since the genetic differences between the lines are the only known causative agents of variation, this variation may properly be attributed to the influence which the inheritance exerts on the utilization of any available vitamin D and of other elements in the deficient diet. The variation within each line may, for the moment, be considered to be induced by unknown environmental factors. The contribution of these two sets of factors to the total variability is seen below.

Males	
Variation in whole population	1,334,400 days
Between related individuals	848,320 days
Within related lines	486,080 days
Females	
Variation in whole population	690,720 days
Between related individuals	377,120 days
Within related lines	313,600 days

These results show that over half of the sum squares of the durations of life within the whole population are due to the differences between the genetically different lines of rats. For the males the contribution made to the whole variation by these racial differences amounts to 63 percent of the total. For the females the contribution is 55 percent of the total. The statistical significance of these differences may be determined in the usual way by comparing the variance of the between group class with that of the within group class, where the degrees of freedom are 10 for between lines and 159, males, and 156, females, for within the lines. These variances for the males are, between lines 84,832 days; and within lines 3,057 days; and for the females, between lines 37,712 days; and within lines 2,010 days. The variance due to the genetic differences is at least 18 times that within the racial lines where a difference of only 2.5 times would be significant. Both sexes thus agree in showing a well marked difference in the effects of the vitamin D deficient diet in populations of closely related individuals as contrasted with a group bred at random. The differences between the variances of the males and females, while fairly large could easily be due to random sampling since to be significant one difference would need to be more than 5 times the other.

There are three classes of genetic relationship for the individuals within the different racial lines. The first is that between individuals of the same litter; the second that between individuals of the same parents but different litters; the third that between the offspring of one pair with those of another pair of the same line. The variance contributed to the total by these different groupings of the data is found below.

These results are concordant in showing the closely related individuals to be quite similar in their duration of life whether they are born into the same litter, different litters of the same parentage or born of different parents but of the same racial inbred line. The results for the males vary somewhat, those for the females are rather constant, seeming to represent a more nearly average result. The variance within the matings—2,200 days for the males and 1,672 days for the females—is a fair estimation of the random variance unaccounted for by the differences in the racial lines. As pointed out earlier, the ratio between this variance and that between the racial groups shows that statistically speaking the odds in favor of an inherited effect on a dietary exhaustion are large indeed. Environmental effects common to the progeny of different parents within racial lines or to litters of the same parents also contribute elements tending to make their duration of life more nearly alike. If the variance of these two groups be compared with that of the within litters group, we find that to be considered significant, the former value should be almost twice the latter. Three out of four groups meet this requirement. The variance of one of the three groups, between litters of the same parents in the males, is nearly three times that of the within litters group. These differences may be considered as indicative of some effect of common environmental or genetic factors (uncontrolled by the previous inbreeding) which were common to these particular groups. The net conclusion to be drawn from the evidence is, however, clear; the life-span of rats confined to the deficient diet is influenced markedly by genetic constitution.

	DEGREES OF FREEDOM	SUM OF SQUARES IN DAYS	VARIANCE IN IN DAYS
Males			
Whole population	169	1,334,400	
Between racial lines	10	848,320	84,832
Between different parents but within racial lines	19	82,400	4,320
Between litters of same parents	23	147,120	6,400
Within litters	117	256,560	2,200
Females			
Whole population	166	690,720	
Between racial lines	10	377,120	37,712
Between different parents but within racial lines	19	65,200	3,432
Between litters of same parents	17	47,760	2,808
Within litters	120	200,640	1,672

THE INTERRELATION OF CHARACTERS AFFECTING THE LIFE-SPAN

It is of interest to inquire further into the inheritance effect and to determine, if possible, what characters the inheritance may affect and thus account for the observed differences between the genetic lines. Two such characters which vary with the different inbred lines are known to be present—the size of litter and the ability to reach a given initial weight at 46 days, the age when the deficient diet was commenced. These variables are interrelated. Both could conceivably be responsible for the observed inherited effects if they are found to be correlated with the survival times of the different lines. Furthermore it seems likely that these variables, litter size and weight at 46 days, would themselves be correlated. The correlations are presented below.

Besides the effect on the life-span, four other measures have been used in estimating the effects of the defective diet, maximum weight attained while on the defective diet, weight just prior to death, days between start of diet and maximum weight, and days between maximum weight and death. The interrelations of these variables, as well as their relation to initial weight and litter size, have a direct bearing on the problem in hand.

TABLE 6

Correlations of survival time and other indicated variables for rats on a vitamin D deficient diet

CHARACTERS CORRELATED	CORRELATION COEFFICIENTS	
	MALES	FEMALES
Weight at 46 days and age at maximum weight	0.43± 04	0.29±.05
Weight at 46 days and maximum weight	.82±.02	.83±.02
Weight at 46 days and age at death	.60±.03	.50±.04
Weight at 46 days and last weight	.76±.02	.84±.02
Weight at 46 days and litter size	−.48±.04	−.46±.04
Age at maximum weight and maximum weight	.71± 03	.52±.04
Age at maximum weight and age at death	.85±.01	.76±.02
Age at maximum weight and last weight	.64±.03	.47±.04
Age at maximum weight and litter size	−.23±.05	−.06±.05
Maximum weight and age at death	.78±.02	.68±.03
Maximum weight and last weight	.91±.01	.91±.01
Maximum weight and litter size	−.39±.05	−.28±.05
Age at death and last weight	.67±.03	.58±.03
Age at death and litter size	−.34±.05	−.15±.05
Last weight and litter size	−.33±.05	−.38±.05

Examination of table 6 reveals a close correlation between many of the variables. Such variables as age at maximum weight, maximum weight, last weight, are closely correlated with age at death, the coefficients being quite high, 0.85, 0.78, 0.67, for the males. These variables may be utilized to measure the defects of the diet and they also completely determine such other variables as gain or loss in weight on the deficient diet. This fact makes it entirely proper to confine our consideration of the inheritance to its effects on the life-span, since such other items are in large part only different measures of the same variable. The litter size and individual weight attained at 46 days, the time when the deficient diet was commenced, are in a somewhat different category in so far as their relation to the effects of the diet on the life-span is concerned. Weight at 46 days is rather highly correlated with the after life-span when the rat is on the deficient diet. The ability to reach a given weight at a given time is also an inherited characteristic. The correlations of table 6 suggest that the inheritance of this character is a contributing cause of the observed inheritance of the length of the life-span between the inbred lines. A similar observation may be made for the litter size. This latter case is somewhat different, however, since litter size may be a variable which expresses its effect entirely through its influence on the weight at 46 days. If this be true, all the attention may be given to the weight at 46 days. The partial correlation coefficients throw some light on the problem. The first order partial correlation coefficient between the weight at 46 days and the life-span where account is taken of the effect of the litter size is 0.53 ± .04 for the males, and 0.49 ± .04 for the females. The partial correlation coefficient for litter size and the life-span where the effect of the 46-day weight is properly accounted for is −0.06 ± .05 for the males and 0.11 ± 0.5 for the females, correlations which are not statistically significant. The characteristic which accounts for all of the effect of litter size and also contributes something to determining the life-span under the conditions of the experiments is the weight at 46 days. The contribution which the inheritance of this character makes to the length of survival may now be investigated.

ON THE CHARACTER BASIS FOR THE GENETIC DIFFERENTIATION SHOWN IN THE DURATION OF THE LIFE-SPAN

The inbred strains of rats utilized for these experiments differ markedly in their weights at 46 days of age, the period including nursing, weaning and growth. The broad ration which was used has maintained a colony of many rats throughout life and over a period of 15 years. Of the total squared sums of these weights for the whole population of males, 45 percent is contributed by the line differences. For the females the contribution is 57 percent. These contributions are statistically significant since the variance between the lines is for the males 13 times and for the females 19 times those of the variances within the lines.

The question of how much this inheritance is contributory to the inheritance of the duration of life under the unfavorable diet may be analyzed as follows. The sum of the squared differences for the durations of life of all rats was earlier split into two parts; that which is due to the differences between the inbred lines and that found within the lines. The question to be answered is, how much of each of these variations is due to the variations in initial weight? The numerical values necessary to answer this question may be determined from the correlation coefficients between the weights at 46 days and the life-spans within the two groups, between the inbred lines and within these lines.

These correlation coefficients are equal to 0.84 for the males and 0.62 for the females in the group where the variation is dependent on genetic differences between the inbred lines. Statistically speaking, they are significant. They are not significantly different from each other, however, since the difference is only 0.22 with a probable error of about 0.28. An average value of 0.73 for the correlation coefficient would thus be a fair estimate of the effect of the inheritance of weight on the life-span. The correlation coefficients between weight and duration of life within the inbred lines is the same for both males and females, 0.34. This is also significant since a correlation of only 0.21 would be exceeded by chance but once in 100 trials. This correlation of 0.34 within the inbred lines would seem to be due to two possible kinds of variables; inheritance heterozygosis, which is as yet uncontrolled by the inbreeding, and common environmental factors having common effects on growth and life-span. It is significant to note that correlations due to such causes are only half those due to the controlled genetic causes, showing that to this extent, at least, the inbreeding has segregated important hereditary factors for life-span and weight into fairly pure racial groups.

The amount of the variation in life-span due to differences in the inheritance for weight may be determined from the relation

$$v = V(1 - r^2)$$

when V is the variance of the life span, r is the correlation coefficient between weight at 46 days and the survival time and v the partial variance, that is, the variance remaining after account has been taken of the effect of the inherited variation in weight. By substituting the correlation coefficient of 0.73 in this equation we find that the variance remaining after account is taken of the effect of the weight at 46 days is only 47 percent of that where weight varies as it will. Of that portion of the total variance attributable to inherited differences between the rat strains (59 percent), 53 percent or 31 percent of the total is contributed by inherited differences in weight. The remaining 47 percent of the inheritance effect on duration of life is due to characters which are at present unknown. The same reasoning may be applied to the variation in length of life within the different lines. The variation due to the uncontrolled inheritance of weight within the inbred lines or to common environmental factors is measured by a correlation of 0.34. The variance remaining after proper account is taken of these factors is 88 percent. The portion of the variance within rat strains due to this character, weight, is consequently but 12 percent of that observed within the rat strains. The tabulation of these results is as follows. The total sum of the squares is reduced to a percentage, 100, and the contribution of each of the different factors to this variation listed as a percentage of this total.

Total variation in life span (sum of squares)	100
Variation between genetically different lines	
Percent of variation due to inheritance of weight	31
Percent of variation due to unknown characters	28
Total attributable to inheritance factors	59
Variation within genetically similar lines	
Percent of variation due to weight	5
Percent of variation due to unknown environmental characters	36
Total attributable to chance inherited and environmental influences	41

TABLE 7

Analysis of the variation of weight at 46 days and life-span in strains in inbred rats.

ORIGIN OF VARIATION	DEGREES OF FREEDOM	WEIGHT AT 46 DAYS (GMS.)		LIFE-SPAN (DAYS)		CORRELATION	
		SUM OF SQUARES	VARIANCE	SUM OF SQUARES	VARIANCE	CROSS PRODUCTS	CORRELATION COEFFICIENT
Male							
Whole population	169	58,010	334.3	1,334,400	7,896	166,630	0.599
Between strains	10	26,073	2,607.3	848,320	84,832	124,210	.835
Within strains	159	31,938	200.9	486,080	3,057	42,420	.341
Females							
Whole population	166	37,785	227.5	690,720	4,161	80,710	0.499
Between strains	10	21,655	2,165.5	377,120	37,712	56,350	.624
Within strains	156	16,130	103.5	313,600	2,010	24,360	.343

In general, the evidence leads to the following conclusions. Since the only known variable which tends to differentiate the inbred lines is heredity, the evidence indicates that somewhat more than half (about 59 percent) of the variation in life-span under conditions made unfavorable by a deficiency of vitamin D in the diet, is due to characters under hereditary control. Approximately 36 percent of the variation is due to unknown environmental influences. Of the half due to heredity, a half of that, or a quarter of the whole, is attributable to the inheritance of the character weight at 46 days. The other quarter, although definitely due to heredity, must be attributable to the inheritance of characters unknown but important to survival under the prescribed dietary conditions. Only 5 percent of the variation within the lines is assignable to weight variations and 36 percent to unknown but nevertheless very real environmental or uncontrolled hereditary factors. The data from which these comparisons are drawn are given in table 7.

SOME GENERAL BIOLOGICAL ASPECTS OF THE DATA

The data favor the view that within a species the reactions of individuals to apparently essential dietary constituents may differ and that this variation is controlled partly by the inheritance. It is of interest to digress and consider here the consequence of such variation in evolution. In the shifting of species from one locality to another of quite different dietary possibilities, it would seem that extensive modification in the physiological economy of the group would often be required. Such, for instance, would be the case of groups moving from high, dry plateaux over mountains into low, hot, moist regions; or, in the laboratory, in the transfer of bacteria from a susceptible host to culture media and *vice versa*.

Two explanations for such modifications in evolution seem possible. First the group could move into the unfavorable area and through some action of the environment become modified and survive the conditions imposed. These modifications, of course, would have to be hereditary and the objections can be raised that the germ plasm is not readily altered and that only a small proportion of such modifications as do occur improve the survival value of the animal. The chance of establishing the initial foothold in the unfavorable area would thus be slight. An alternative proposition would be that within a group certain animals may become potentially suited in their genetic constitutions to environments different from those in which they are found, but into which they can migrate. These extreme forms which would rise at random and without any evident adaptive relation to the environment would thus be fitted to meet the new environmental conditions and to establish themselves in sufficient numbers to gain a foothold. Further mutation and selection, even though the rate be small and the effect slight, would eventually tend to complete the adaptive change.

The evidence obtained in our experiments points to distinct genetic differences in the dietary requirements of strains of rats. These differences existed before there was any known exposure of the race to dietary deficiencies. Certain lines were adjusted by their previous inheritance to a somewhat lower requirement of given food elements than were others, and could, consequently, meet the lack of such elements more successfully. The fitting of certain members of the group to the environment came first rather than as a consequence of previous modification.

This view is supported in the experiments on disease resistance, in which the different inbred lines of a race are exposed to a disease rare to it. Examples include pseudorabies of cattle introduced into mice (GOWEN and SCHOTT 1933) or *B. abortus* of swine in rabbits (COLE, 1930), since in both instances it has been possible to demonstrate that certain strains have inherited resistance to the disease, whereas others show distinct susceptibility to it.

Other diseases which are known to be quite dependent upon the genetic constitution of the host for their infectivity do not furnish such critical evidence, since these diseases occur commonly in the species and selection for susceptibility or resistance may have occurred.

Such diseases as rat typhoid, *S. enteritidis* of IRWIN (1929), mouse typhoid *S. aertrycke* of SCHOTT (1932), *B. enteritidis* of WEBSTER (1933), white diarrhea, *S. pullorum* of ROBERTS and CARD (1926), typhoid, *S. gallinarum* of LAMBERT (1932) fall into this group, since it is uncertain whether the fitting of specific groups to the environment came first or as a consequence of previous modification.

The bacteria and especially the protozoa furnish significant evidence of both types of genetic modification of the host organization. Evaluation of the results necessitates a slight reorientation in thought, since the selection may affect the soma instead of the germ plasm in these rapidly reproducing unicellular forms. In certain bacteria, the colon group, for instance, division may occur every 20 minutes. A single organism then, in the course of 8 hours could give rise to 16,000,000 individuals if all survived during the period. Since the possible rate of mutation for a specific gene is 1 to 10 in 10,000,000 individuals nearly every gene and the character or characters which it governs could have changed at least once in 24 generations.

Wild species of paramecium (JENNINGS 1908), difflugia (JENNINGS 1916, 1929), centropyxis (ROOT 1918), yeast (HANSON and others 1906), colon bacillus (BARBER 1907), and many others have shown several biotypes of diverse heredity, each of which is capable of reacting differently to the same or to diverse environments. Experimentally, the environmental agents used were in some cases poisons with which the organism presumably had had no previous contact,—the parasitic trypanosomes (TALIAFERRO 1926) or the free-living paramecia (JOLLOS 1921).

The choice of the original biotype seemed fortunate in certain cases (JENNINGS 1929) since the results were not always reproducible with other stocks. The effects followed a pattern closely similar to that observed in other less prescribed environments, in which for example the criterion was that the animals should reach a given size or survive a certain heat. The initial contrast between the selected lines seemed to be due to previously differentiated biotypes selected from the wild population. Further selection and presumably further purification of these biotypes resulted in further progress, that is, increase in size or resistance to the heat or poison. If still further progress was attained it occurred in more or less discrete steps of unequal length in both generation time and advance toward the greatest adaptibility for the environment. This goal, as DALLINGER (1887) showed, may be far removed from the ordinary environmental conditions under which the species are accustomed to live: in 7 years time the temperature requirements of flagellates were changed from 60°F. to 158°F., 16°C. to 70°C. But these changes in the genetic structure of the organisms are no more permanent than those found in the wild type from which they started many generations earlier. For, if the environment is now changed, the forms may again, by a slow step-like process, be made to return to forms whose requirements are those of the wild stock from which it originated. This result is not so surprising if cognizance is taken of the relatively large numbers of animals involved, and the rates at which genes vary under natural conditions It suggests that in any population, as in the rat population here studied, variation in the nutritive requirements, etc., are occurring which are purely outside the requirements of the given environment and that if the environment should change those variants having the capacity to meet the changed conditions become the parents of those which are to form the new race.

SUMMARY

The data presented here show that rats which are fed a low vitamin D, high calcium diet, from the 46th day of age have an average duration of life of 5 to 6 months, or not quite half, that of like animals on a normal diet. Rats on the deficient diet die off more sharply than those fed normally. The standard deviations of duration of life of rats on the deficient diet are less than half those of animals on the normal diet. The relative variation is consequently greater in the normal rats. It is further noted that males on the vitamin D deficient diet live slightly longer than the females, but on a normal diet the results are reversed. The frequency curves of death show a distinct skewness.

Data for the separate lines show distinct differences between these lines in respect to length of life. The variations range from 131 to 345 days for the males and 132 to 267 days for the females. A comparison of the variations found for the whole random bred population and for the rats related by strain, shows that a distinct correlation exists between the duration of life of the animal within a given line. The correlation coefficient for the males is 0.63; that for the females 0.55.

Within the strains there are 3 relationships which bear on the strain difference: correlations between members of the same litter, between individuals of two litters and the same parents, and between individuals of

the same line but of different parents. The analyses of the effects of these relationships are concordant in showing that the genetic differences between the racially differentiated lines account for the greater part of the variation. Variation due to the similarity in environment of offspring of the same parents or of the same litter are rather small and statistically just significant.

The hereditary differences between the inbred strains are found to account for somewhat more than half of the variations observed in the life-span. Of this hereditable variation, about half seems to be due to the inheritance of the character, body weight at 46 days. The remaining quarter of the variation in life-span due to heredity may be attributed to the inheritance of characters as yet unknown but important to survival under the dietary conditions imposed by the experiment. Of the variation remaining after account is taken of the inheritance effect, only 5 percent is attributable to variation in weight. The other 36 percent is due to unknown factors apparently largely of environmental origin, although even here some of this variation could be due to some heterozygosis of the germ plasm as yet uncontrolled by the inbreeding.

Finally, the evidence considered from a broader biological viewpoint has a bearing on the problems of adaptive evolution which may be significant. The suggestion is made that the fitting of certain groups within a species to particular environmental conditions would seem to come first rather than as a consequence of modification by the environment. The experiment presented above supports such a contention, for the evidence points to distinct genetic differences in the dietary requirements of the strains which, so far as it is known, never had been exposed to the particular deficiencies involved.

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THE GENETICS AND PHYSIOLOGY OF SELF-STERILITY IN THE GENUS CAPSELLA

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INTRODUCTION

THE oppositional factor hypothesis of EAST and MANGELSDORF (1925) and FILZER (1926) will account for the genetics of self-sterility in many species of plants, particularly those of the Solanaceae and Scrophulariaceae. When two cross-fertile plants are mated normally, either four intra-sterile, inter-fertile classes will result all of which will be fertile with both parents, or only two such classes will be produced of which one will be fertile with both parents while the other will be fertile with the mother and sterile with the father; in either case, the classes will be of the same size. The class of the mother is never represented in the offspring. Therefore, reciprocal crosses are never alike. Self-sterility in these species is controlled by three or more allelic genes, usually designated s^1, s^2, s^3, etc. Two only are present in the tissues of the style of a normal diploid plant; and when a pollen grain which bears either one of the two is placed upon the stigma, its pollen tube does not grow sufficiently rapidly to reach the ovules before the flower withers. On the other hand, when a pollen grain which carries a self-sterility gene different from either one in the style is placed upon the stigma, growth of the tube proceeds at an accelerated rate and fertilization takes place.

There are other species of plants which are self-sterile but in which the behavior of the intra-sterile, inter-fertile classes is different from that in the species of the Solanaceae and Scrophulariaceae which have been investigated. The author (RILEY 1932) showed that *Capsella* (*Bursa*) *grandiflora* definitely does not behave as it should if the same type of *s* factors controlled self-sterility. In that paper, a possible genetic scheme was suggested, but further work indicates that it must be modified. CORRENS (1912) had described a case of self-sterility in *Cardamine pratensis*, a plant which belongs to the same family as Capsella. He found that two cross-fertile plants produced four intra-sterile, inter-fertile classes; one was sterile with the mother and fertile with the father, another was just the opposite, a third was fertile with both parents and the fourth was sterile with both. The results in Capsella which were reported earlier seemed to indicate an analogy with the situation in Cardamine. In most families, only the two classes of the parents were found, but in others, a third class fertile with both parents was present; some families seemed to contain a class sterile with both parents in addition to the two parental classes, while others contained all four classes, as CORRENS had found in Cardamine. An occasional family produced only one class which further complicated the situation. It seemed at the time, that the situation in these two Crucifers was basically the same, but that some modifying condition was present in Capsella; it was the purpose of the further investigation reported in this paper to determine what was the nature of this modifying condition.

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Another species which proved to be different from Nicotiana and Veronica and therefore not to be controlled by oppositional factors was *Lythrum salicaria*. DARWIN, BARLOW, EAST and VON UBISH all found that the three types of flowers in this species were relatively intra-sterile; and the latter workers showed that in most plants two gene pairs determine trimorphism. In Lythrum, self-sterility seems to be determined by the same genes *A*, *a*, *B*, and *b* which determine the three forms of the flowers.

MATERIALS AND METHODS

The plants used in this investigation resulted from crosses reported in the previous paper, and the technique of hand pollination was the same. The families of the first generation of these new studies were raised in Doctor G. H. SHULL'S greenhouse at PRINCETON UNIVERSITY, while those of the next two were grown in the greenhouse of the BUSSEY INSTITUTION of HARVARD UNIVERSITY through the courtesy of Doctor E. M. EAST, under whose sponsorship this work was carried out, and under a fellowship from the NATIONAL RESEARCH COUNCIL.

In the earlier paper, the three intra-sterile, inter-fertile classes were designated by the letters A, B, and C, as in tables 3 and 4 of that paper. The method of identifying classes from one generation to another also was given. The classes considered in this paper descended from those of the first paper and the same symbols are used for the three classes. There is an unbroken continuity of classes in the two papers.

OBSERVATIONS

In the previous paper, certain families seemed to be composed of three classes, one of which was sterile with both parents, while some other families consisted of four classes of which one again seemed to be sterile with the classes of both the mother and the father. There was no *a priori* ground for doubting this situation, and as the latter type of family was identical with that found by CORRENS, the tentative explanation offered previously was based upon the assumption that this situation was correct. When families appeared in subsequent generations which seemed to split in the same manner, however, the plants which gave sterile results with both testers were retested. When the two testers were again crossed on to plants which were sterile with both parents, and if the second tests were made a few weeks after the first trials, it was discovered that these plants set seed with one of the test plants. For example, plant 62(6) had been crossed with plant 62(1) and set no seeds in nine capsules; with 62(5) no seeds were produced in six capsules. As plants 62(1) and 62(5) were fertile together and therefore of different classes, plant 62(6) seemed to be sterile with both testers. Plants 62(1) and 62(5), by appropriate tests, were shown to be of the same classes as their parents, so that family 62 seemed to segregate into three classes of which two resembled the parents while the third was sterile with both parents. The cross, 62(6)×62(1), had been made in the beginning of December 1931 when the plants had just begun to bloom, and the other cross, 62(6)×62(5), had been made a few weeks later. On March 1, these crosses were repeated when the plants were mature but still in good condition. The cross 62(6)×62(5) again yielded no seeds in seven pollinations, while the other cross, which had previously been classed as sterile, produced 45 seeds in five pollinations, or an average of nine seeds per capsule. After this was established, all plants which seemed to be sterile with both parents and which could be retested were retested later, and in all cases one of the crosses which had been sterile proved to be fertile upon repetition. It was impossible, of course, to repeat crosses upon plants which had given similar results in previous generations, but it is probable that if such could be done, these plants also would prove to be fertile with one of the testers and therefore with one of the parents. This discovery threw a new light upon the situation. Just what determines this early sterility in such cases has not been determined, but it is probably not due to self-sterility. It is reasonable, then, to reexamine the data, discarding as insufficiently tested all plants in the earlier generations which seemed to be sterile with both parents. When this is done, it is seen that these families split into two groups which are like the parents or into three of which two are like the parents and the third fertile with both parents, or that only one class is present and that it is sterile with one parent and fertile with the other. For example, family 2 of the previous paper seemed to be identical with CORRENS's family, segregating into four classes; but if the one plant which was sterile with both testers is disregarded, the family splits into three classes of five, two, and four plants, respectively.

When all the families reported previously are reconsidered, assuming that plants recorded as sterile with both parents appear to be so because of a technical error, it is seen that most families segregate into two groups, but that four families consist of all three groups and that these families came from the cross A×C. It was the first purpose of this investigation to trace such families further.

Family 39 was one family that split into three classes, and intra-family crosses were tested the following year. Family 62 from the cross 39(5)×39(1), or Class B×Class A, contained five plants of Class A and five of Class B. Family 63 arose from 39(9)×39(1). Plant 39(9) was Class C, and this cross resulted in four plants of Class A, four of Class C and one of Class B. Family 64, from 39(9)×39(5), contained five plants of Class C and five of Class B. The parents were Classes C and B. Family 39, then, consisted of classes A, B, and C. Class A×Class B gave the same two classes as the parents, and the same was found to be true when the parents were of Classes B and C. However, when Classes A and C were intercrossed, a family was produced which consisted of all three classes.

Although this result began to make the situation much clearer, it was considered advisable to study the classes which are produced when self-sterile segregates from crosses between self-sterile and self-fertile plants are crossed with other plants of the self-sterile species. This point was tested in 1932. Families 69, 70, 71 and some plants of 72 served as test material to identify the classes of these hybrids.

Family r3 was the F_1 between a Class C plant of *C. grandiflora*, 26502(7), and a plant of the self-fertile species *C. rubella*. Self-fertility is dominant to self-sterility (RILEY 1934), and four plants of family r3 were selfed to produce an F_2. All four resulting families split. The self-steriles of three of these families were all of Class B, while those of the fourth, r6, were Class C. A heterozygote of family r6 was selfed to produce family r24. Plant r24(15), a self-sterile of Class C, was crossed with 63(6), which was of Class A, reciprocally. The resulting families, r35 and r37, split into Classes A and C. When r24(15) was crossed with a Class B plant, twelve plants of Class C resulted and none of Class B.

A cross between a Class B plant, 26502(6), and the self-fertile *C. tuscaloosae* resulted in an F_3 family, t26, in which all the self-sterile segregates were of Class B. Plant t26(3) was crossed reciprocally with plant 60(1) (Class C) and Classes B and C were present in each resulting family.

To omit a lengthy discussion of the families, most of the families of *C. grandiflora* and of hybrids between this species and the three self-fertile species which are discussed in this and the previous paper are listed in table 1. This table includes the origin of the families, the genotypes of the parents according to the theory outlined in the next section of this paper, and the classes expected and those obtained in the offspring.

DISCUSSION

Before this more recent work, it appeared that self-sterility in Capsella was determined by the same type of gene that appears to control the situation in Cardamine, but that balanced lethals with crossing-over

TABLE 1

Listing the families of Capsella grandiflora and some of the families of the self-fertile species and hybrids of the entire study, with their origin in terms of self-sterility genes and the expected and obtained classes.

FAMILY	PARENTS	GENOTYPES OF PARENTS	CLASSES EXPECTED	CLASS OBTAINED
1	26674(18)×26674(13)			7A:4B
2	26669(50)×26669(49)			5A:4C:2B
3	26664(35)×26664(31)			2A:1C
4	26502(7)×26502(6)	$ttS^{c}s \times ttss$	1C:1B	1C:4B
5	26502(6)×26502(7)	$ttss \times ttS^{c}s$	1C:1B	4C:2B
7	2(2)×1(1)	$ttS^{c}S^{c} \times Ttss$	1A:1C	5A:3C
8	2(10)×1(11)	$ttS^{c}s \times ttss$	1C:1B	7C:3B
9	5(3)×2(12)	$ttss \times Ttss$	1A:1B	7A:4B
10	2(2)×1(2)	$ttS^{c}S^{c} \times ttss$	All C	10C
11	2(12)×1(11)	$Ttss \times ttss$	1A:1B	6A:4B
12	2(10)×1(1)	$ttS^{c}s \times Ttss$	2A:1C:1B	Insuff tested
13	5(3)×2(10)	$ttss \times ttS^{c}s$	1C:1B	2C:7B
14	2(4)×1(2)	$ttS^{c}s \times ttss$	1C:1B	8C:6B
15	5(6)×4(5)	$ttS^{c}s \times ttss$	1C:1B	8C:2B
17	5(5)×2(12)	$ttS^{c}s \times Ttss$	2A:1C:1B	3 classes
18	5(6)×2(12)	$ttS^{c}s \times Ttss$	2A:1C:1B	3 classes
29	3(3)×1(1)	$ttS^{c}S^{c} \times Ttss$	1A:1C	2 classes
30	3(3)×2(7)	$ttS^{c}S^{c} \times ttss$	All C	10 C
31	4(3)×1(11)	$ttS^{c}s \times ttss$	1C:1B	3C:7B
33	5(1)×4(5)	$ttS^{c}s \times ttss$	1C:1B	6C:4B
34	8(1)×8(5)	$ttS^{c}s \times ttss$	1C:1B	4C:5B
35	8(5)×8(1)	$ttss \times ttS^{c}s$	1C:1B	4C:6B
36	9(2)×12(1)	$ttss \times Ttss$	1A:1B	6A:4B
37	12(1)×9(2)	$Ttss \times ttss$	1A:1B	5A:5B
38	9(6)×10(1)	$Ttss \times ttS^{c}s$	2A:1C:1B	5A:3C:1B
39	10(1)×9(1)	$ttS^{c}s \times Ttss$	2A:1C:1B	8A:1C:1B
42	8(5)×9(1)	$ttss \times Ttss$	1A:1B	6A:4B
43	8(5)×14(9)	$ttss \times ttS^{c}s$	1C:1B	3C:7B
44	9(2)×9(1)	$ttss \times Ttss$	1A:1B	8A:2B
45	9(3)×9(1)	$ttss \times Ttss$	1A:1B	5A:5B
49	5(3)×2(12)	$ttss \times Ttss$	1A:1B	6A:4B
51	7(4)×7(1)	$ttS^{c}s \times TtS^{c}s$	4A:3C:1B	3A:5C
52	7(9)×7(1)	$ttS^{c}s \times TtS^{c}s$	4A:3C:1B	3A:7C
53	2(2)×1(1)	$ttS^{c}S^{c} \times Ttss$	1A:1C	7A:2C
56	2(10)×1(11)	$ttS^{c}s \times ttss$	1C:1B	7C:3B
59	10(1)×9(2)	$ttS^{c}s \times ttss$	1C:1B	3C:7B
60	35(2)×35(5)	$ttss \times ttS^{c}s$	1C:1B	4C:3B
61	36(3)×36(4)	$Ttss \times ttss$	1A:1B	5A:4B
62	39(5)×39(1)	$ttss \times Ttss$	1A:1B	5A:5B
63	39(9)×39(1)	$ttS^{c}s \times Ttss$	2A:1C:1B	4A:4C:1B
64	39(9)×39(5)	$ttS^{c}s \times ttss$	1C:1B	5C:5B
65	42(2)×42(1)	$Ttss \times ttss$	1A:1B	7A:3B
68	59(2)×59(1)	$ttS^{c}s \times ttss$	1C:1B	3C:3B
69	60(2)×60(1)	$ttss \times ttS^{c}s$	1C:1B	2C:5B
70	61(3)×61(1)	$Ttss \times ttss$	1A:1B	5A:5B
71	62(1)×62(5)	$Ttss \times ttss$	1A:1B	7A:3B
72	63(4)×63(1)	$ttS^{c}s \times Ttss$	2A:1C:1B	insuff. tested

TABLE 1. (*Continued*)

FAMILY	PARENTS	GENOTYPES OF PARENTS	CLASSES EXPECTED	CLASSES OBTAINED
3333	60(7)×60(1)	$ttss \times ttS^cs$	1C:1B	4C:6B
3334	61(8)×61(3)	$ttss \times Ttss$	1A:1B	5A:4B
3335	69(2)×69(1)	$ttS^cs \times ttss$	1C:1B	8C:2B
3336	70(9)×70(1)	$Ttss \times ttss$	1A:1B	10A:1B
3337	2(11)×1(11)	$TtS^cS^c \times ttss$	1A:1C	4A:2C
3338	2(12)×1(11)	$Ttss \times ttss$	1A:1B	5A:3B
3342	39(3)×39(5)	$TtS^cs \times ttss$	2A:1C:1B	1A:9C:10B
3343	39(4)×39(5)	$Ttss \times ttss$	1A:1B	6A:10B
3344	39(6)×39(5)	$Ttss \times ttss$	1A:1B	8A:7B
3345	39(7)×39(5)	$Ttss \times ttss$	1A:1B	3A:10B
3348	63(9)×63(2)	$Ttss \times ttS^cs$	2A:1C:1B	5A:12C
3349	70(3)×69(2)	$Ttss \times ttS^cs$	2A:1C:1B	12A:3C:5B
r3	26502(7)×26322	$ttS^cs \times ttS^fS^f$	self-fertile	self-fertile
r7	r3(2)×self	$ttS^fs \times$ self	self-steriles = B	1B identified
r6	r3(4)×self	$ttS^fS^c \times$ self	self-steriles = C	9C
r8	r3(5)×self	$ttS^fs \times$ self	self-steriles = B	1B identified
r12	r3(3)×self	$ttS^fs \times$ self	self-steriles = B	1B identified
r24	r6(56)×self	$ttS^fS^c \times$ self	self-steriles = C	1C identified
r30	r24(3)×self	$ttS^fS^c \times$ self	self-steriles = C	6C identified
r32	r24(5)×self	$ttS^fS^c \times$ self	self-steriles = C	20C identified
r34	r24(9)×self	$ttS^fS^c \times$ self	self-steriles = C	3C identified
r35	63(6)×r24(15)	$Ttss \times ttS^cS^c$	1A:1C	4A:2C
r36	r24(15)×64(1)	$ttS^cS^c \times ttss$	All C	12C
r37	r24(15)×63(6)	$ttS^cS^c \times Ttss$	1A:1C	3A:2C
3350	r32(1)×70(7)	$ttS^cS^c \times ttss$	All C	24C
3351	r32(1)×r35(1)	$ttS^cS^c \times TtS^cs$	1A:1C	14A:6C
t1	26502(6)×26323	$ttss \times ttS^fS^f$	self-fertile	self-fertile
t12	t1(3)×self	$ttS^fs \times$ self	self-steriles = B	1B identified
t26	t12(16)×self	$ttS^fs \times$ self	self-steriles = B	8B identified
t36	t26(11)×self	$ttS^fs \times$ self	self-steriles = B	1B identified
t37	t26(13)×self	$ttS^fs \times$ self	self-steriles = B	10B identified
t38	t26(15)×self	$ttS^fs \times$ self	self-steriles = B	1B identified
t47	60(1)×t26(3)	$ttS^cs \times ttss$	1C:1B	6C:4B
t48	61(3)×t26(3)	$Ttss \times ttss$	1A:1B	4A:4B
t49	t26(3)×60(1)	$ttss \times ttS^cs$	1C:1B	4C:2B
t50	t26(3)×61(3)	$ttss \times Ttss$	1A:1B	4A:2B
3352	t48(1)×t49(4)	$Ttss \times ttS^cs$	2A:1C:1B	10A:3C:5B
v1	26502(7)×26324	$ttS^cs \times ttS^fS^f$	self-fertile	self-fertile
v2	v1(2)×self	$ttS^fs \times$ self	self-steriles = B	9B identified
v3	v1(6)×self	$ttS^fS^c \times$ self	self-steriles = C	7C identified

might account for the small numbers of certain classes in some families. To check this point, counts were made of the number of seeds and of aborted ovules per capsule and of seed germination to learn whether 50 percent seed abortion due to balanced lethals took place. The percentage of viable seed to aborted ovules was extremely variable (from about 16 percent to 90 percent) and there were no indications that balanced lethals were in operation.

The realization that certain classes seemed to be sterile with both parents because of an error in technique, particularly due to pollination when the female plant was too young, made untenable any theory analogous to that of CORRENS; another explanation was sought. To account for self-sterility in Capsella, it must be borne in mind that the self-sterile species is a diploid ($n=8$) and not a tetraploid, as are *C. bursa-pastoris*, the common species of Shepherd's-purse, and most other species of the genus. *C. rubella*, *C. tuscaloosae*, and *C. Viguieri* are also diploids. Chromosome counts by HILL (1927) and MANTON (1932) established this, but confirmation for this material was sought by examining a number of plants during the last few generations. All showed that the 2n number is 16. The chromosomes are small, and good meiotic figures are few and difficult to obtain, but the diploid nature of these plants is unmistakable.

In formulating an explanation for the genetic situation in Capsella, several important results must be considered. In the first place, when one of the parents was of Class A, this class always appeared in the progeny. This was not true of Class B, for B×C gave only C in several cases of rather large families. Further, when Class B was crossed to a self-fertile, the F_2 self-sterile segregates were all of Class B, while Class C, when mated with a self-fertile, produced F_2 families in which all the self-steriles were either Class B or Class C. These observations can be accounted for by supposing that the sporophytic nature of any two plants determines whether they will be mutually sterile or fertile, and that self-sterility and cross-sterility are controlled by two pairs of genes. The best assumption is that all plants of Class A are sterile together because they possess a dominant gene, *T*. Any two plants having this gene will be reciprocally cross-sterile, but any plant which lacks *T* and is therefore *tt* will be fertile with any plant which has *T*. From this it is seen that *T* must always exist in a heterozygous state, since a plant bearing it can set seed with *tt* plants only. Classes B and C must be *tt* in order to be fertile with Class A, and the differential in this case is another pair of genes which will be designated S^c and *s*. The gene *T* is epistatic to these others. In the absence of *T*, plants which bear the dominant gene of this second pair will be reciprocally cross-sterile and will belong to Class C. Finally, the ultimate recessive, *ttss*, will represent the genotype of Class B plants. In the absence of either dominant gene the combination *ttss* acts to produce incompatibility with all other plants of the same genetic constitution.

If this assumption be correct, Class A×Class B and the reciprocal will reproduce the same two classes if the genotypes of the parents are *Ttss* and *ttss*. However, the plants of Class A may bear the gene S^c in homozygous or heterozygous condition since it is hypostatic to *T*, and if a plant of that nature is mated with one of Class B, Class C will appear in the progeny. In some cases, Class B × Class C produced both parental classes, while in several families, only Class C was found. This would be expected if the Class C plants had as their respective genotypes ttS^cs and ttS^cS^c. If a Class A plant which is TtS^cs is mated with a plant of Class C (either ttS^cS^c or ttS^cs), the cross will be fertile in spite of the presence of the S^c gene in both plants, since T is epistatic to S^c and completely nullifies the action which the S^c gene would have in the absence of T. Since T cannot be obtained in a homozygous state, there are six possible genotypes which represent the three intra-sterile, inter-fertile classes in Capsella. The results obtained from all the possible combinations of these genotypes are shown in table 2.

TABLE 2

Intra-sterile inter-fertile classes of C. grandiflora with their genotypes and the results of all the possible combinations between these genotypes.

	CLASS A			CLASS C		CLASS B
	TtS^cS^c	TtS^cs	$Ttss$	ttS^cS^c	ttS^cs	$ttss$
TtS^cS^c	S	S	S	F	F	F
TtS^cs	S	S	S	F	F	F
$Ttss$	S	S	S	F	F	F
ttS^cS^c	F	F	F	S	S	F
ttS^cs	F	F	F	S	S	F
$ttss$	F	F	F	F	F	S

There are eleven possible combinations of these genotypes which will be fertile, excluding reciprocal crosses, since they always give the same results. Of these possibilities, six had been tested before 1933. The possible combinations and the families which illustrate them are tabulated in table 3. The only families which were tested sufficiently and which did not split into the classes expected according to the nature of their parentage were families 51 and 52. These arose from crosses within family 7 which in turn came from the cross 2(2) × 1(1), or Class C × Class A. Plant 2(2), when mated with Class B [plant 1(11)] produced family 10, which was composed of Class C plants only, so that plant 2(2) must have had the constitution ttS^cS^c. When mated with 1(1), it should have produced Classes A and C in family 7, which it did; these plants should have been TtS^cs and ttS^cs. When two cross-fertile plants of family 7 were crossed, the offspring should have been Classes A, B and C. In both 51 and 52, however, only A and C were found. The only explanation that can be offered is that when the two test plants of the families were crossed onto their sibs, the one or two plants which were of Class B were too young and appeared to give sterile results with the A or C testers instead of being fertile with both. The cross 51(4)×51(1) produced one seed in seven capsules and possibly would have been fertile had the cross been made later in the life of the plant. Had this occurred, plant 51(4) would have been recognized as belonging to Class B. The same is true of plants 52(5) and 52(8). The complicating sterility condition mentioned earlier in this paper is probably the reason why only two classes were identified in these two families.

TABLE 3

Possible combinations of genotypes giving fertile results, the genotypes and phenotypes of the offspring and all the families which illustrate them.

PHENOTYPES OF CROSS	GENOTYPES OF CROSS	GENOTYPES OF OFFSPRING	PHENOTYPES OF OFFSPRING	FAMILIES
A×B	$TtS^cS^c \times ttss$	$TtS^cs + ttS^cs$	A+C	3337;
A×B	$TtS^cs \times ttss$	$TtS^cs + Ttss + ttS^cs + ttss$	A+B+C	3342;
A×B	$Ttss \times ttss$	$Ttss + ttss$	A+B	1; 9; 11; 36; 37; 42; 44; 45; 49; 61; 62; 65; 70; 71; t48; t50; 3334; 3336; 3338; 3343; 3344; 3345;
A×C	$TtS^cS^c \times ttS^cS^c$	$TtS^cS^c + ttS^cS^c$	A+C	none;
A×C	$TtS^cS^c \times ttS^cs$	$TtS^cS^c + TtS^cs + ttS^cS^c + ttS^cs$	A+C	none;
A×C	$TtS^cs \times ttS^cS^c$	$TtS^cS^c + TtS^cs + ttS^cS + ttS^cs$	A+C	3351;
A×C	$TtS^cs \times ttS^cs$	$TtS^cS^c + TtS^cs + Ttss + ttS^cS^c + ttS^cs + ttss$	A+B+C	2;
A×C	$Ttss \times ttS^cS^c$	$TtS^cs + ttS^cs$	A+C	7; 53; r35; r37;
A×C	$Ttss \times ttS^cs$	$TtS^cs + Ttss + ttS^cs + ttss$	A+B+C	18; 38; 39; 63; 3349; 3352;
B×C	$ttss \times ttS^cS^c$	ttS^cs	C	10; 30; r36; 3350;
B×C	$ttss \times ttS^cs$	$ttS^cs + ttss$	B+C	4; 5; 8; 13; 14; 15; 31; 33; 34; 35; 43; 56; 59; 60; 64; 68; 69; t47; t49; 3335

In order to test the validity of the theory outlined above, an additional generation was raised in 1933–34, with the families so set up as to produce definitely predictable classes. Families 3333, 3334, 3335 and 3336 were used as test families, producing Classes A, B, and C which were used to identify the classes of the other families. Family 3337 came from the cross 2(11)×1(11), or Class A×Class B. As these seeds were old, the percent of germination was poor and only six plants reached maturity. Four were Class A and two were Class C, which would indicate, in spite of the small population, that plant 2(11) was TtS^cS^c. This is quite probable if one examines the origin of family 2. Tests of plant 2(12) had indicated that it was *Ttss* (Class A). A cross between this and the Class B plant, 1(11), produced family 3338. From its origin, it was expected to split into Classes A and B only. There were five plants of Class A and three of Class B. A similar set-up produced families 3343, 3344 and 3345. In all these families, the Class B parent was plant 39(5), which, it will be remembered, belonged to a family which had segregated into all three classes. When crossed with three plants of Class A, plant 39(5) produced only Classes A and B in families 3343, 3344 and 3345.

Family 3342 is interesting. The parents were 39(3) and 39(5), or Class A and Class B. Twenty plants were raised, and tests showed that one was of Class A, ten of Class B and nine of Class C. When one parent of a cross is Class A, this class invariably appears in the progeny because of the epistasis of the gene T which is present in plants of Class A. The small size of this class in family 3342 was probably the result of test pollinations upon Class A plants in this family when the plants were too young to give their mature sterility reactions. The splitting of this family into all three classes from the cross of A × B indicates that the plant of Class A which was used as a parent had the genetic constitution $TtS^{c}s$, which is perfectly in accord with expectations considering the origin of family 39.

The only family of this series which did not give the expected classes was family 3348. The parents of this family were 63(9) (Class A) and 63(2) (Class C). From the origin of family 63, the plants of Class A had the genetic constitution of $TtS^{c}s$ or $Ttss$, while all Class C plants were $ttS^{c}s$. Irrespective of which type of plant of Class A was selected, the progeny should have produced all three classes, A, B, and C. In family 3348, however, there were five plants of Class A and twelve of Class C, but none of the other expected class, B. Undoubtedly, this absence of plants of Class B is due to the action of this peculiar complicating sterility which has been described. The presence of Class C in such unexpectedly large numbers indicates that some plants classed as belonging to this class were probably Class B plants which showed sterility with the Class C tester because of this juvenile sterility. There was no time to repeat the crosses. It is this complicating condition which has made an analysis of genetics of self-sterility in this species so difficult.

Plant 70(3) was a plant of Class A which must have been homozygous for the gene s, according to the origin of the family. This was crossed with plant 69(2), a plant of Class C with the constitution $ttS^{c}s$. Such a cross should have produced Classes A ($TtS^{c}s$ and $Ttss$), B ($ttss$), and C ($ttS^{c}s$). This cross produced family 3349, and tests upon this family showed that there were twelve plants of Class A, five of Class B, and three of Class C. This was the expected result, and the numbers of each class are not far from the theoretical, which would be 10A:5B:5C.

Two families used for these corroboratory tests were from crosses involving the F_4 self-sterile segregate r32(1). Family r32 originated from a selfing of the heterozygous self-fertile plant r24(5) which had come down from a cross between *Capsella grandiflora* (Class C) and a plant of *C. rubella.* Twenty self-sterile segregates of family r32 were all of Class C and the heterozygous parent was therefore ttS^fS^c. Self-fertility is dominant to self-sterility. The evidence for the location of the factor for self-fertility is not so conclusive as it would be had self-fertile plants been crossed with self-steriles of Class A, but from the data on hand, it can be assumed that this gene is an allele of the S^c-s series and dominant to both S^c and s. Also, it seems to be epistatic to the gene T. Plant r32(1) was undoubtedly ttS^cS^c as it is a self-sterile segregate from the self-fertile plant whose genetic constitution was ttS^fS^c. Plant r35(1) (Class A) has been shown to have come from a self-sterile segregate of Class C crossed with a pure *C. grandiflora* plant of Class A, and plant r35(1) was TtS^cs in constitution. A cross between it and r32(1) produced family 3351 which split into fourteen plants of Class A and six of Class C as expected. When the same female plant, r32(1) was crossed by a plant of Class B, 70(7), only plants of Class C should be expected, and they should have the genes ttS^cs. Family 3350 arose from such a cross and was composed of 24 plants all of which were of Class C.

The final family which was examined in this generation was 3352, and it resulted from seeds of the cross t48(1) × t49(4). Family t48 resulted from a cross between a plant of Class A which was homozygous for s and a self-sterile segregate which was $ttss$. Therefore, plant t48(1), since it was of Class A, had the constitution $Ttss$. Family t49 came from the same plant of Class B which produced t48 and a plant of Class C, which had the constitution ttS^cs. Plant t49(4) was ttS^cs. When the cross t48(1) × t49(4) was made, it was predicted that the offspring would split into three classes, since $Ttss \times TtS^cs$ should give Class A (TtS^cs and $Ttss$), Class B ($ttss$), and Class C (ttS^cs). In family 3352, there were ten plants of Class A, five of Class B and three of Class C, which is not far from the expected numbers 9, 5 and 4.

These more recent tests show that the explanation of a two-factor situation with one gene epistatic to the other pair and of the determination of self-sterility, cross-sterility and cross-fertility by the sporophytic nature of both parents is a tenable theory. As regards the genetics of self-sterility in *C. grandiflora*, the same type of genes appears to be present as have been found to control both heterostyly and self-sterility in *Lythrum salicaria* (EAST 1927). In the latter case, the genes A, a, B and b produce two results, one morphological (heterostyly) and the other physiological (self-sterility). The same kind of genes in Capsella, however, has no morphological effect and produces only self-sterility.

In this study on Capsella, the size of the individual families is small, but in several cases, the same type of cross produced a number of families. In table 3, the families produced by the various combinations of genotypes are listed. The summation of the numbers in each class in all the families of several matings are cited below.

$Ttss \times ttss$	(22 families)	= 130A:97B.	Expected	113.5:113.5
$Ttss \times ttS^cS^c$	(4 families)	= 19A: 9C.	Expected	14 : 14
$Ttss \times ttS^cs$	(5 families)	= 39A:14C:13B.	Expected	33 : 16.5:16.5
$ttss \times ttS^cs$	(20 families)	= 92C:87B.	Expected	89.5: 89.5

While the deviations from the expected are not large, it is suggestive that the double recessive class, *ttss*, is below expectation in all three types of mating where it has appeared. Class A, which contains the epistatic factor, *T*, exceeds expectation in the three types of mating in which it is involved.

PHYSIOLOGY OF SELF-STERILITY

In the previous paper to which frequent reference has been made (Riley 1932), it was suggested that self-sterility was due to a failure of the pollen to germinate in an incompatible mating. Further studies corroborate this finding. In fertile crosses, the pollen tubes can be seen leaving the pollen grains and passing among the large cells at the top of the stigma; it is difficult to trace the tubes further, but there is no doubt that the pollen grains have germinated. In a sterile combination, the picture is very different. About ninety-nine percent of the pollen grains fail completely to germinate; those that do germinate, send out short tubes which never elongate and which flatten out against the cells of the stigma without growing down among them, or which in some instances grow away from the stigma as is illustrated in figure 1.

In Nicotiana, the pollen germinates in all self- and cross-pollinations. In fertile combinations, the rate of pollen-tube growth is accelerated by chemical substances secreted by the pistil (East and Park 1918), while in sterile matings, the growth curve is depressed after twenty-four hours (East 1934). In *Petunia violacea*, Yasuda (1934) showed that self-sterility is caused in part by the failure of pollen to germinate, but chiefly by differential pollen-tube growth.

East (1934) maintains that substances are present in the stigma of the mature flower which react with substances in the pollen tubes, resulting in a slowing up of the growth of the tubes in a sterile combination. In most plants, these substances are not present in the young bud, and appear during the twenty-four hours just preceding the opening of the flower. In certain self-sterility genotypes, these substances are developed even earlier, and no bud pollinations can be procured. The behavior of pollen grains in self-sterile matings of Capsella is different from the situation in Nicotiana, but this difference is probably not a fundamental one. In Nicotiana (EAST 1934), these substances appear to be present in a certain region of the stigma. When the tubes reach this place, their growth-rate slows up markedly, but after this zone of interference is passed, the growth curve again approaches a straight line. In Capsella, it seems that the same sort of reaction occurs, but that the zone of interference is located at the very end of the stigma in the stigmatic hairs. Instead of slowing up pollen tubes which had begun active growth, the stigmatic substances are produced at such a place in the pistil that the grains do not germinate or at most produce tiny, abortive tubes. It is probable that not only are these substances located higher in the stigma, as the non-germination of most of the pollen grains would indicate, but that they are more powerful, for even when a few tubes do germinate, they cannot enter the stigma.

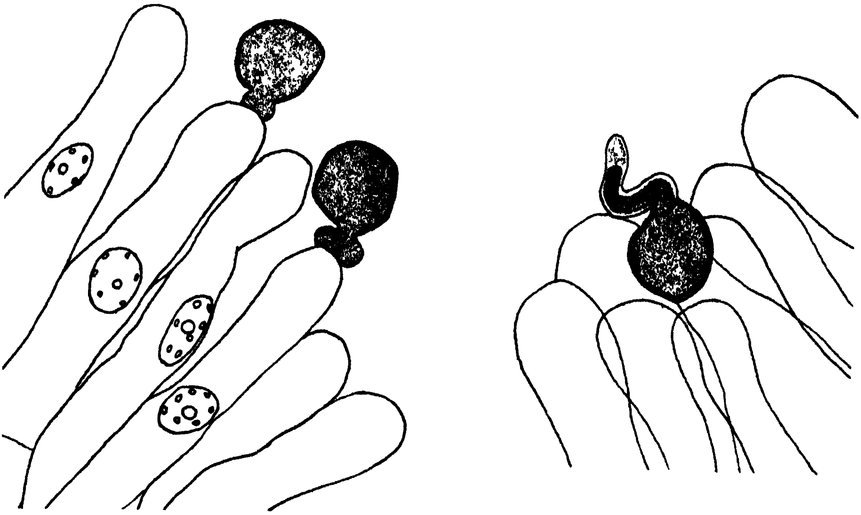

FIGURE 1.—Stigmatic hairs of *Capsella grandiflora*, showing abnormal pollen germination in incompatible matings· (Left) pollen tubes flattened against the stigmatic hairs; (Right) a pollen tube growing away from the stigma. Such cases are exceptional, for most pollen grains do not germinate in an incompatible combination. Aceto-carmine smears, drawn with camera lucida, at table level with 40 objective and 15× ocular.

In Nicotiana, self-sterility is determined by the diploid or sporophytic nature of the female and the haploid or gametophytic nature of the male. If a plant has the genes s^1 and s^2, secretions reflecting their nature would be present in the tissues of the style. If the pollen grains from a plant whose genetic constitution is s^1s^3 are placed upon this stigma, the s^3 grains send out tubes which are accelerated in growth and which eventually fertilize the ovules. The grains which bear the s^1 gene also germinate, but there is a reaction between the pollen-tubes which come from them and the substance in the style which is produced by the s^1 gene in the female plant. This reaction has a depressive effect on the growth-rate of the tubes. The tubes which bear the s^1 gene are solely s^1 in nature. The pollen mother cells of this s^1s^3 plant were s^1s^3 and the pollen grains have either the s^1 or s^3 genes. Their pollen-tubes are either s^1 or s^3 in reaction. It is reasonable to assume that when the nuclear membrane disappeared during the first division of the microspore bearing the s^1 gene, substances secreted by this gene diffused out into the cytoplasm so that the cytoplasm shows the reactions characteristic of this gene.

In *Capsella grandiflora*, self-sterility, cross-sterility, and cross-fertility are determined by the sporophytic nature of both the male and female plants, as is also true of Lythrum. If a plant has the genes ttS^cS^c, it behaves like a plant of Class C and is sterile with all other plants bearing the genes tt and S^c in combination. When a Class C plant of the genetic constitution ttS^cs undergoes reduction, the microspores are either tS^c or ts. If the pollen grains of this plant are placed upon the stigma of the ttS^cS^c plant, those whose constitution is tS^c fail to germinate, but those which are ts also do not germinate in spite of the absence of the S^c gene in their make-up. On the other hand, pollen grains bearing the genes ts and which came from the B Class ($ttss$) will germinate readily. A plant with the constitution TtS^cS^c will behave as a plant of Class A. If this is crossed by another plant of Class A, the genotype of which is $Ttss$, the result will be sterility although the individual pollen grains of this second plant will be either Ts or ts. The ts pollen, on the other hand, from a $ttss$ or a ttS^cs plant will germinate on and produce seeds with the plant TtS^cS^c. The pollen mother cells of the $Ttss$ plant will undergo reduction to form grains of which the genes will be Ts or ts, but the cytoplasm of these grains will react alike. It is plausible to assume, then, that at the first meiotic division, when the nuclear membrane is broken down, substances produced by the T gene disseminate into the cytoplasm so that at telophase the nuclei are Ts or ts as to their genes, while the cytoplasm of all the cells is T in its reacting properties. This cytoplasmic nature persists throughout the ensuing divisions after the first meiotic division, so that all the male gametophytes have cytoplasm which reacts like that of the sporophyte from which the gametophyte developed. For that reason, the pollen grains which have only ts genes but which came from a sporophyte of the constitution $Ttss$ react like the Ts pollen grains from the same sporophyte, but react entirely differently from ts pollen grains from a $ttss$ sporophyte. The nature of the reacting cytoplasm of pollen grains in Capsella, therefore, is determined probably by a release of nuclear material during the last cell division of the sporophyte generation and the persistence of its ability to function.

SUMMARY

1. Three intra-sterile, inter-fertile classes only have been found in *Capsella grandiflora* and are designated as Classes A, C and B.

2. Class A×Class C will produce Classes A and C or Classes A, B and C, but never Classes A and B only. Class A×Class B will produce Classes A and B or Classes A, B and C or Classes A and C. Class B×Class C will produce Classes B and C or Class C only, but never a plant of Class A. Reciprocal crosses are always alike and produce the same classes in every case.

3. When a plant of *C. grandiflora* of Class B is crossed with a self-fertile species, all the self-sterile segregates in the F_2 are of Class B. When Class C is mated with a self-fertile plant, the self-sterile F_2 plants in some families will be Class C and in others will be Class B, but all the self-steriles in any one family are of the same class.

4. Self-sterility appears to be due to two pairs of genes, Tt and S^cs. T is epistatic to S^c. All Class A plants bear the gene T and are sterile together reciprocally, irrespective of the nature of the other genes. Since fertile results will be produced only when a T plant is crossed with tt, no plants homozygous for T are found. Class C plants are all tt and bear the gene S^c which may be homozygous or heterozygous. All plants with either one or two doses of this gene will be mutually inter-sterile. Plants of Class B are $ttss$ and are all sterile together. The sporophytic nature of the two plants entering into a cross determines whether or not seeds will be produced. From available data, self-fertility appears to be caused by a gene S^f which forms a third member of the allelic series S^f-S^c-s and which is dominant to either of the other two genes. It also appears to be epistatic to the gene T.

5. Pollen grains bearing the genes ts will be sterile upon a plant of the nature TtS^cS^c if they came from a plant whose genetic constitution was $Ttss$ or TtS^cs, but will be fertile upon TtS^cS^c if the plant from which they were derived was $ttss$ or ttS^cs. This and other evidence indicate that there is a release of nuclear material into the cytoplasm during the reduction division of the pollen mother cells and which shows its effect in the cytoplasm of the mature pollen grain.

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CYTOGENETICS OF HYBRIDS BETWEEN ZEA MAYS AND EUCHLAENA MEXICANA[1]

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INTRODUCTION

UNDOUBTEDLY specific and generic as well as lesser differences may be largely attributed to differences in the chromosomes. Detailed analysis of gene and chromosome differences between related genera is seldom possible because of sterility and lack of genetical or cytological information. A striking exception is provided by *Zea mays* L. and *Euchlaena mexicana* Schrad. Between these distinct species highly fertile hybrids are easily obtained. Genetic and cytological work with maize is sufficiently advanced to make analysis of hybrids profitable.

Linkage groups as well as cytological markers have been established for all 10 chromosomes in *Zea mays*. In some cases the approximate position of genes on a chromosome has been determined. Some of the most useful chromosome markers have been obtained as a result of the recent work with segmental interchange strains. The meiotic prophase crosses and rings which appear in maize heterozygous for an interchange make identification of the chromosomes concerned relatively easy.

A consideration of these facts led to the belief that hybrids of maize and annual teosinte would be exceptionally favorable material for cytogenetical studies. From the study of hybrids it was hoped that a clearer knowledge of the similarities and differences of the chromosomes of maize and its relative, annual teosinte, would be obtained. Knowledge of this kind is useful in estimating the evolutionary processes which have been effective in the differentiation of the two forms studied.

Three varieties of annual teosinte are recognized: Florida, Durango, and Chalco. The latter two varieties have been found growing wild in Mexico (Collins, 1921), whereas the cultivated Florida variety is thought to have come originally from Guatemala. Recently, Collins (1932) has reported that an annual teosinte resembling the Florida variety has been discovered which is native to that region.

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Morphologically, *Euchlaena mexicana* differs markedly from *Zea mays* especially in ear characters. BEADLE (1932) reports that the Florida variety is the only one which, when crossed with maize, produces noticeably sterile hybrids. The variety Durango, used to a lesser extent in our crosses, produces hybrids which appear to be almost completely fertile. A rather surprising fact reported by BEADLE (1932) is that hybrids between the Florida and Durango varieties are highly sterile, about 67 percent of the pollen grains being deficient in starch. In this case inter-varietal sterility is greater than the inter-generic sterility found when either of these varieties is crossed with maize.

All 3 varieties of *E. mexicana* have the same chromosome number ($n=10$) as has *Zea mays*. The morphological features of the chromosomes of the Florida variety are similar to those of maize (BEADLE, 1932), except for the presence of terminal knobs on 8 of the 10 chromosomes. As in maize, a satellite chromosome is present.

MATERIALS AND METHODS

To test the pairing relations of particular maize chromosomes with corresponding teosinte chromosomes, hybrids were obtained between teosinte and maize strains homozygous for known reciprocal translocations. A descriptive terminology introduced by ANDERSON (1935) is now used in denoting the various reciprocal translocations in maize. Thus, T1-2a is homozygous for the reciprocal translocation between chromosomes 1 and 2 which was first described. T1-2b would apply to a second translocation involving the same 2 chromosomes. Ordinary maize will be referred to in this paper by the old term *o*-normal to distinguish it from translocation types.

MCCLINTOCK (1930), COOPER and BRINK (1931) and others have shown that in maize heterozygous for an interchange involving 2 chromosomes, the 2 interchange chromosomes and the 2 "normal" ones with which they pair form a characteristic cross-shaped figure at pachytene stages of the first meiotic division. The cross complex opens out at diakinesis into a ring of 4 chromosomes. The formation of these cross-shaped complexes has added to the proof that prophase pairing of chromosomes is determined by homology of the pairing strands.

It was expected that structural differences between maize chromosomes and their respective teosinte "homologs" in hybrids would be revealed by modification of the structure of the cross-shaped complex, and by reduction in number or absence of chromosome rings at diakinesis and equatorial plate stages. In each such hybrid, pairing of both ends of 2 or more chromosomes is tested simultaneously. A list of the translocation stocks of maize used is given in table 1.

Anthers were fixed in various modifications of Carnoy's solution. COOPER'S (COOPER and BRINK 1931) modification (2 parts chloroform: 2 parts glacial acetic:6 parts absolute or 95 percent alcohol) gave satisfactory results. After fixation for 2–10 hours, the material was washed in two changes of 95 percent alcohol and preserved in 80 percent or 70 percent alcohol. The best smear preparations were obtained from anthers freshly placed in 80 percent or 70 percent alcohol.

TABLE 1

Translocation stocks of maize used in crosses with teosinte.

MAIZE STOCK NEW TERMINOLOGY	CHROMOSOMES INVOLVED IN TRANSLOCATION	DESCRIBED BY
T1-2a	1 and 2	Brink 1927. Cooper and Brink 1931.
T1-6a	1 and 6	Cooper and Brink 1931.
T1-2aT1-6a	1, 2 and 6	Brink and Cooper 1932.
T1-7a	1 and 7	Burnham 1930.
T5-7a	5 and 7	from Anderson—not described.
T4-8	4 and 8	from Anderson—not described
T8-9a	8 and 9	Burnham 1930. McClintock 1930, 1931. Creighton and McClintock 1931.
T1-2aT8-9a	1 and 2 8 and 9	Burnham 1930.

For the smear preparations, iron-aceto-carmine of almost full strength was used. Occasionally dilution by the addition of a little 45 percent glacial acetic was found desirable. Slight pressure was applied to the cover slip and the slide gently heated several times over an alcohol lamp as recommended by MCCLINTOCK (1930).

Crosses between linkage-tester stocks of maize and Florida and Durango teosinte were made to determine crossover values in the hybrids. The F_1 hybrids were backcrossed to the maize parent type in most cases and crossover percentages determined from the character of the backcross generation.

At the latitude of Madison, Wisconsin (43°), the Florida and Durango varieties of teosinte do not flower under field conditions until late September or early October. Plants from vernalized seeds, treated as recommended for maize by LYSSENKO (see WHYTE and HUDSON 1933) did not flower appreciably earlier than untreated plants. Short-day treatment as described by EMERSON (1924), begun May 15 on plants 1 month old was effective, however, in hastening the flowering period. Both Florida and Durango teosinte so treated began shedding pollen in the first week of August. However, this was not early enough to allow all the desired crosses to be made.

CYTOLOGICAL OBSERVATIONS ON F_1 HYBRIDS BETWEEN MAIZE AND ANNUAL TEOSINTE OF THE VARIETIES FLORIDA AND DURANGO

O-normal maize×Florida teosinte

No lack of pairing could be detected in the late pachytene stages. At diakinesis and at equatorial plate stages (plate 1A) an unequal open, or end-to-end, pair (a, a′), sometimes appearing as 2 univalents, could be distinguished in slightly over 50 percent of the cells (table 5). A somewhat larger unequal pair was detected in 20 percent of the cells.

In describing microsporocytes from similar hybrids Beadle (1932) found diakinesis pairing regular except for the presence of 2 unequal univalents in about 50 percent of the cells examined. Less frequently he observed 4 univalents. Our results are in agreement. Two heteromorphic pairs are present in maize-Florida hybrids. The members of these pairs fail to remain attached, or are attached only at one end, in an appreciable proportion of the microsporocytes at diakinesis and equatorial plate stages.

O-normal maize×Durango teosinte

In the relatively few figures examined, no univalents were found. One or 2 end-to-end pairs, perhaps indicating a somewhat reduced chiasma frequency for those pairs, were present in about 25 percent of the cells examined (plate 1B, a, b).

Chromosome 6 of maize, in maize-teosinte hybrids

The sixth chromosome of maize can be recognized in a high proportion of meiotic prophase figures by the satellite at one end by which the chromosome is attached (or appressed) to the nucleole. In hybrids of maize with Florida teosinte pairing of the sixth maize chromosome with a corresponding teosinte chromosome is complete. Attempts were made to count the number of chiasmata present. The counts given in table 2 were made from diakinesis stages. Chiasmata were found to be not confined to any restricted region of the chromosome. If chiasmata are related to genetic crossing over, either as cause or effect, then in hybrids of Florida teosinte and maize, crossing over in this chromosome should be a rather common phenomenon.

Relatively few counts have been made on chiasma frequency in the sixth chromosome in maize-Durango hybrids. As with Florida hybrids, however, the modal number appears to be 2. The figures given here for chiasma frequency may be too low, since in the case of interstitial chiasmata it is often impossible to determine whether more than 1 is present. The lower figure was recorded in such doubtful cases. A size difference, if there is any, could not be established as existing between the sixth chromosome

EXPLANATION OF PLATE 1

The figures were drawn with the exception of M with a Spencer compens 20× ocular and a 2 mm Leitz achromatic objective N.A. 1.32, at table level with the aid of an Abbe camera lucida. With a tube length of 160 mm a magnification of about 2720× was obtained. The figures have been reduced two-thirds in reproduction.

A: O-normal maize×Florida teosinte. Equatorial plate stage. 9 bivalents and 2 unequal univalents (a, a′).

B: O-normal maize×Durango teosinte. Diakinesis stage. 10 pairs of chromosomes. 2 pairs end-to-end (a, b).

C: T1–2a maize×Florida teosinte. Diakinesis stage. T1–2a ring of 4 chromosomes, 7 pairs and 2 unequal univalents (a, a′). The components of one of the end-to-end pairs (b) appear to be unequal in size.

D: T1–2a maize×Florida teosinte. Equatorial plate. Ring of 4, 7 pairs and 2 unequal univalents (a, a′).

E, F, G: T1–2aT1–6a maize×Florida teosinte.

E: Diakinesis. Ring of 6 chromosomes and 7 pairs, including 2 heteromorphic pairs, a and b.

F: Equatorial plate stage. Ring of 6 chromosomes and 7 pairs, including two heteromorphic pairs, a and b.

G: Diakinesis. Ring of 6 drawn from 4 different cells.

H, I: T1–7a×Florida teosinte.

H: Diakinesis stage. Ring of 4 chromosomes (c), and 8 pairs. One open heteromorphic pair (b); and one end-to-end unequal pair (a).

I: Diakinesis stages. Rings drawn from 4 different cells. Lower, (d), open ring. The other 3 rings (a, b, c) are representative examples of the most frequently occurring ring type.

J: T5–7a×Florida teosinte. Diakinesis stage. Chain of 4 chromosomes (b), 7 pairs and 2 unequal univalents (a, a′).

K: T1–2aT8–9a maize×Florida teosinte. Diakinesis. T1–2a ring (c), T8–9a ring (b) and 6 pairs. 1 pair open, unequal (a).

L: T8–9a maize×Florida teosinte. Diakinesis. T8–9a ring (c) and 8 pairs. One pair end-to-end (a); 1 open, heteromorphic pair (b).

M, N, O: T1–2aT8–9a maize×Durango teosinte.

M: Diakinesis. T1–2a ring (a); T8–9a ring (b); and 6 pairs. ×1575.

N: Diakinesis. Typical T1–2a and T8–9a configurations are shown in this cell. T1–2a ring of 4 chromosomes (c). T8–9a as 2 end-to-end pairs (a, b). 6 other pairs of chromosomes.

O: Diakinesis stages. T8–9a configurations. (a) ring, (b) loose chain, (c) 2 "pairs," the most frequently observed type of association.

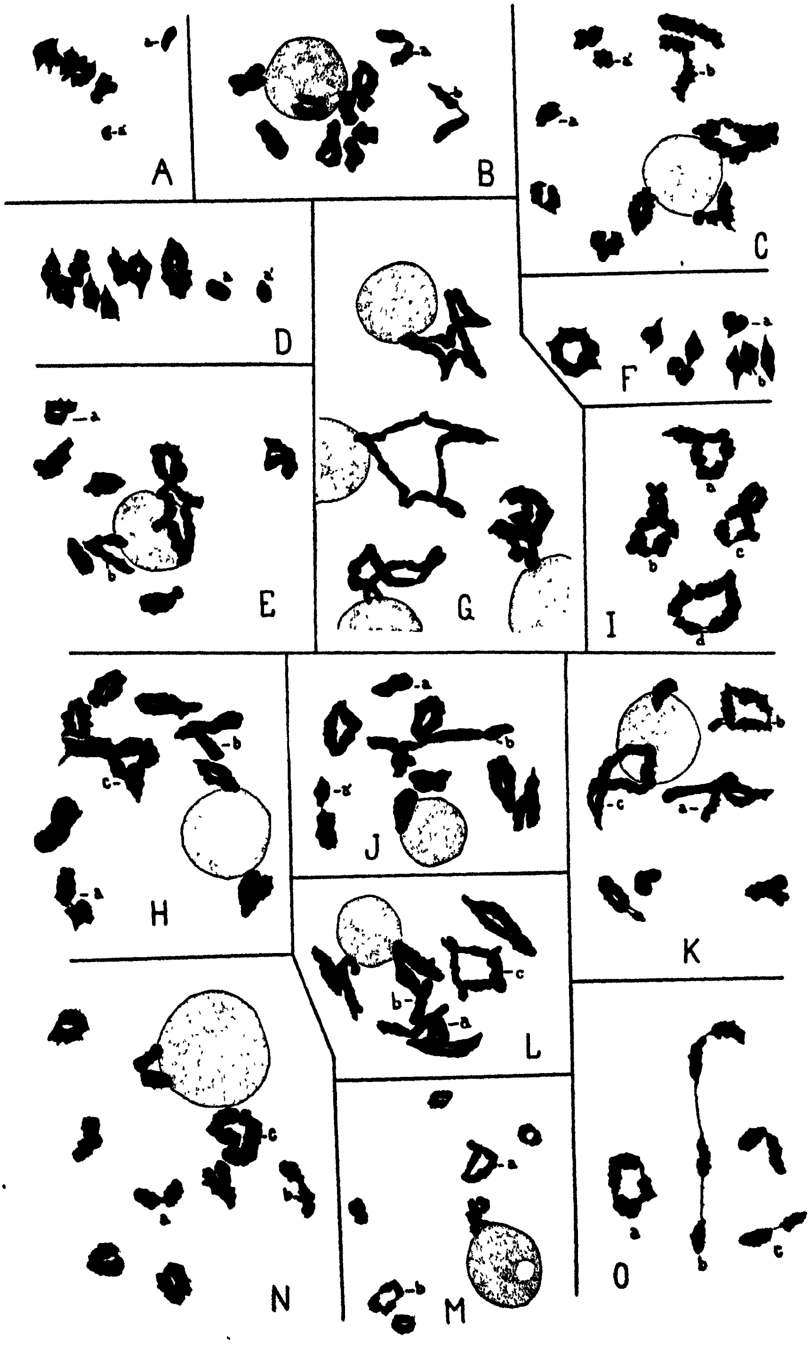
A
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TABLE 2

Distribution of chiasmata in the 6th chromosome in F_1 hybrids of maize and teosinte (diakinesis figures).

	NO. OF PLANTS	0 CHIASMA	1 CHIASMA			2 CHIASMATA				3 CHIMASMATA				TOTAL CELLS
			PROX.	INTER	DISTAL	PROX. AND DISTAL	PROX. AND INTER	DISTAL AND INTER	2 INTER	PROX. INTER AND DISTAL	PROX. AND 2 INTER	DISTAL 2 INTER	3 INTER	
Maize×Florida	10	1	4	9	5	22	7	22	2	15	0	3	1	91
Maize×Durango	2	0	1	2	13	27	3	3	1	1	0	0	0	51

---

[1] Proximal refers to the satellite end of the chromosome.

of maize and the corresponding chromosome of either Florida or Durango teosinte. No lack of pairing could be observed.

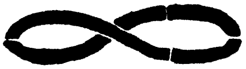
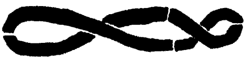
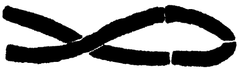
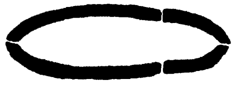
T1–2a maize×Florida teosinte

The *P-br* and *B-lg* chromosomes, the 2 longest of maize, have exchanged segments in the T1–2a strain. Prophase crosses which were usually not marked by any striking peculiarities were observed in the microsporocytes of the hybrids. At diakinesis and at the equatorial plate stages chromosome rings (plate 1C, D) were found in 90.2 percent of the cells counted (table 4).

Both unequal pairs were to be seen in figures in which the ring was present (plate 1C, a, b). The cytological evidence indicates then that the first and second chromosomes of maize pair closely with their Florida "homolog," and that neither chromosome 1 nor 2 is involved in either of the 2 heteromorphic pairs.

TABLE 3

Diakinesis configurations of the interchange complex in T1–7a maize×Floirda teosinte hybrids.

CONFIGURATION	DESCRIPTION	DIAGRAM	NUMBER
ring	1 or more interstitial chiasmata in long chromosomes		73
ring	1 interstitial chiasma in long, 1 interstitial chiasma in short chromosomes		3
ring ring	"distal" ends of long chromosomes free but one interstitial chiasma open-chromosomes held together only at the ends		6
chain			29 7
		Total	118

T1–6a maize×Florida teosinte
T1–2aT1–6a maize×Florida teosinte

Few cells of T1–6a maize×Florida teosinte hybrids were examined. Diakinesis rings of 4 chromosomes appeared to be common, the ring being attached, at early stages, to the nucleole.

Brink and **Cooper** (1932) showed that in the T1–2aT1–6a strain chromosome 1 has exchanged different segments with chromosomes 2 and 6. As would be expected from the previously recorded observations on pairing of chromosomes 1, 2, and 6 of maize in hybrids with Florida teosinte, the predominant diakinesis configuration was a ring of 6 chromosomes (plate 1G) and 7 pairs (plate 1E, F). Since both heteromorphic pairs (a, b) could still be distinguished the chromosomes involved in them are none of those in the T1–2a,T1–6a ring of 6. The high proportion of diakinesis rings, 88 percent (table 4), provides further evidence of intimate pairing of chromosomes 1, 2, and 6 of maize with the corresponding chromosomes of Florida teosinte.

T1–7a maize×Florida teosinte

The strain of maize designated as x-normal-3 by Burnham (1930) is homozygous for a reciprocal translocation between the first and seventh chromosomes. Prophase crosses involving the 2 interchange chromosomes of maize and 2 from the teosinte parent were not found in the few pachytene stages observed in microsporocytes of the hybrids. Diakinesis rings (plate 1H, c, I) were present in 94 percent (table 4) of the cells examined at those stages. A large proportion of the rings (plate 1I, a, b, c) were of a form which indicated that one or more interstitial chiasmata were present in the 2 long chromosomes. Counts of the different types of rings were made (table 3) to determine the approximate proportion of cells having the semi-closed, or figure-8 type of ring.

Since 62 percent of the microsporocytes had chromosome rings of the general type described, pollen counts were made to determine whether an effect on fertility could be discerned. Such an effect might be expected if the prevailing ring configuration affected anaphase distribution of the ring chromosomes, favoring either the passage of alternate or adjacent chromosomes to the same pole. Over 60 percent of the pollen grains examined were deficient in starch, indicating that fertility, if changed at all, is lowered rather than raised by the predominant ring type present at the diakinesis stages in the microsporocytes of these hybrids.

Both unequal pairs of chromosomes (plate 1H, a, b) were also distinguishable in diakinesis figures of these hybrids (table 5) indicating that the seventh maize chromosome is not involved in either pair.

T5–7a maize×Florida teosinte

The fifth and seventh maize chromosomes have exchanged segments in the T5–7a stock. Microsporocytes from only 1 hybrid plant have been obtained as yet. Diakinesis rings consisting of 4 chromosomes were present in 73 percent of the diakinesis figures examined. The larger, less-frequently observable unequal pair was not distinguishable in any of the cells but the smaller, more markedly unequal pair was noted (figure 1 and plate 1J, a, a′) in a number of instances. Too little material has been examined,

TABLE 4

The number and proportions of the diakinesis translocation configurations found in maize-teosinte hybrids.

HYBRID	MAIZE TRANSLOCATION CHROMOSOMES	RING		CHAIN		2 PAIRS		TRIVALENT AND UNIVALENT		PAIR AND 2 UNIVALENTS		OTHER CON-CONFIGURATIONS		TOTAL
		NO.	PERCENT	NO.	PERCENT	NO.	PERCENT	NO.	PERCENT	NO.	PERCENT	NO.	PERCENT	
T1-2a×Florida	1 and 2	55	90.2	5	8.2	1	1.6							61
T1-6a×Florida	1 and 6													
T1-2aT1-6a×Florida	1, 2 and 6	164	88.2	21	11.3							chain of 4 and 1 pair 1	0.5	186
T1-7a×Florida	1 and 7	111	94.1	7	5.9									118
T5-7a×Florida	5 and 7	37	72.6	7	13.7	7	13.7							51
T4-8×Florida	4 and 8	5	15.6	18	56.2	2	6.3	7	21.9					32
T8-9a×Florida	8 and 9	36	53.7	17	25.4	13	19.4			1	1.5			67
T1-2aT8-9a×Florida	1 and 2	207	89.2	20	8.6	5	2.2							232
	8 and 9	118	50.9	61	26.3	34	14.7	8	3.4	11	4.7			232
T1-2aT8-9a×Durango	1 and 2	97	92.4	6	5.7	2	1.9							105
	8 and 9	7	6.7	12	11.4	77	7.33	1	1.0	8	7.6			105

however, to be conclusive. The meager data suggest that in this hybrid ring formation is somewhat reduced, and that the larger of the 2 heteromorphic pairs may be included in the translocation complex in the hybrid.

T4–8 maize × Florida teosinte

The interchange complex in microsporocytes of T8–9a maize × Florida teosinte hybrids frequently takes the form of 2 "pairs." It is consequently of interest that in T4–8 maize × Florida teosinte hybrids a chain of 4 is most frequently observed. Other configurations occur (table 4), though less frequently. The appearance of the T4–8 complex has not been figured in the literature, but presumably rings of 4 are most common. It is suggested that a segment of the 8th maize chromosome frequently fails to be attached by a chiasma to a corresponding teosinte chromosome segment. The smaller unequal pair of chromosomes was noted in about 30 percent of the microsporocytes (table 5).

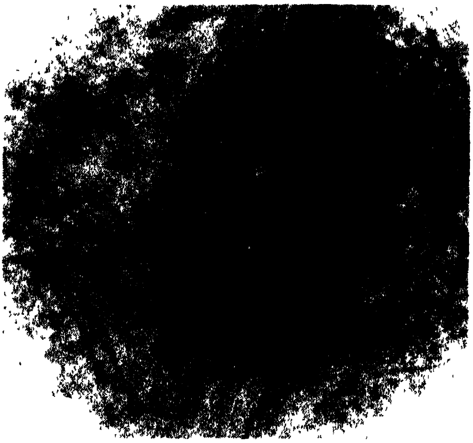


FIGURE 1.—T5–7a maize × Florida teosinte. Equatorial plate stage. Ring of 4 chromosomes, 7 pairs, and 2 unequal chromosomes. The unpaired chromosomes are off the plate. Photomicrograph. 850×.

T8–9a maize × Florida teosinte
T1–2aT8–9a maize × Florida teosinte
T1–2aT8–9a maize × Durango teosinte

The point at which the translocation break has occurred in the ninth chromosome in T8–9a maize has been established by a combination of cytological and genetical methods (BURNHAM 1930; MCCLINTOCK 1931; CREIGHTON and MCCLINTOCK 1931). In the T1–2aT8–9a strain of maize the 2 translocation chromosomes of the T1–2a stock are also present. The

TABLE 5

Numbers and proportions of heteromorphic pairs of chromosomes in diakinesis figures of maize-Florida teosinte hybrids.

HYBRID	NUMBER OF PLANTS	SMALL UNEQUAL		MEDIUM UNEQUAL		TOTAL CELLS	PERCENT SMALL UNEQUAL	PERCENT MEDIUM UNEQUAL
		2 UNIVALENTS	"PAIR"	2 UNIVALENTS	"PAIR"			
0-normal maize ×Florida teosinte	7	44	41	7	23	150	56.7	20.0
T1-2a maize ×Florida teosinte	2	21	5	2	9	61	42.6	18.0
T1-2aT1-6a maize ×Florida toesinte	2	23	36	2	32	186	31.7	18.3
T1-7a maize ×Florida teosinte	2	11	3	1	3	31	45.1	12.9
T5-7a maize ×Florida teosinte	2	6	12	0	0	51	35.3	—
T4-8 maize ×Florida teosinte	2	3	7		2	32	31.2	6.2
T8-9a maize ×Florida teosinte	4	?	?	0	8	67	—	11.9
T1-2aT8-9a maize ×Florida teosinte	5			7	30	180	—	20.6

T8–9a interchange complex in hybrids of these translocation stocks by Florida teosinte takes the form of a ring at diakinesis (plate 1K, b, L, c) in approximately one-half of the cells counted. A chain of 4, 2 "pairs," a trivalent and one univalent, and a pair and 2 univalents were the other observed configurations (table 4). At least 1 of the heteromorphic pairs (plate 1K, a) was also present (table 5). In the T1–2aT8–9a maize×Durango teosinte hybrids the T1–2a ring (plate 1M, a, N, c) was present at diakinesis in over 90 percent of the cells examined; the T8–9a ring (plate 1M, b, O, a) in only 6.7 percent (table 4).

In both Durango and Florida teosinte hybrids with T8–9a, the interchange complex, at diakinesis, takes the form of a ring of four chromosomes much less frequently than it does at corresponding stages in pure maize heterozygous for the translocation, that is, 97 percent according to BEADLE (1932). If ring formation depends on the formation of 1 or more chiasmata in each arm of the prophase cross, then the very marked reduction in number of rings formed in these hybrids indicates failure of chiasma formation in 1 or more arms of the pachytene cross in a rather high proportion of cases.

SUMMARY OF CYTOLOGICAL RESULTS

A summary of the general results is presented in tables 4 and 5. The available cytological evidence indicates close correspondence of chromosomes 1, 2, 6, and 7, somewhat less for chromosome 5, of maize with Florida teosinte chromosomes. The reduced number of rings in T8–9a maize×Florida teosinte hybrids suggests that a structural difference preventing chiasma formation exists between the eighth or ninth chromosomes, or both, of maize and the Florida teosinte chromosomes with which they pair, at least partially. Even less correspondence of these chromosomes in Durango hybrids is indicated. Cytological evidence of approximately normal pairing between chromosomes 1, 2 and 6 of maize with Durango teosinte chromosomes has been obtained.

CYTOGENETIC STUDIES INVOLVING THE NINTH MAIZE CHROMOSOME IN MAIZE-TEOSINTE HYBRIDS

The genes used, together with their position in the linkage group as given by EMERSON (1932) are listed below.

yg_2	—yellow green seedling and plant	; at locus 0
sh	—shrunken endosperm	; at locus 22
wx	—waxy endosperm	; at locus 52

The spindle attachment region is believed to lie near the *wx* locus. The translocation point lies some 12 genetic units from *wx* on the long arm of

TABLE 6

Backcross data on crossing over between chromosome 9 of maize and the corresponding chromosome of Florida teosinte.

INVESTIGATOR	TEOSINTE	$Yg_2\text{-}Sh$	$Yg_2\text{-}sh$	$yg_2\text{-}Sh$	$yg_2\text{-}sh$	$Sh\text{-}Wx$	$Sh\text{-}wx$	$sh\text{-}Wx$	$sh\text{-}wx$	TOTAL
Beadle	Florida	551	3 0.3%	0 0.0%	475	550	1 0.1%	0 0.0%	478	1029
Arnason	Florida	569	14 1.5%	10 1.0%	366					959
Arnason	Florida					978	42 2.0%	9 0.4%	1031	2060

the chromosome (Beadle 1932). No genes are known in the long arm.

yg_2 sh wx maize was crossed with Florida teosinte, which carries the corresponding dominant allelomorphs. The hybrids were then backcrossed to the recessive parent. The backcross seeds and plants were classified with the results as given in table 6, where they are compared with those of Beadle (1932).

Because of poor germination of backcross seeds, more than twice as many seeds were classified for the endosperm characters *sh* and *wx* as could be classified for the plant character yg_2. The results of the two independent investigations summarized in table 6 are in agreement to the extent that both show crossing over to be much less frequent in the short arm of the ninth chromosome in maize-Florida teosinte hybrids than in maize.

A comparison of Beadle's results with those of the writer reveals that the crossover values obtained by the latter are higher for both yg_2–*sh* and *sh*–*wx* regions of the ninth chromosome.

The plants classified as $\frac{Yg_2\text{–}sh\text{–}wx}{yg_2\text{–}sh\text{–}wx}$ or $\frac{yg_2\text{–}Sh\text{–}Wx}{yg_2\text{–}sh\text{–}wx}$ were grown to maturity to check their classification. Ten authentic crossovers were established. The remainder proved to be non-crossovers and may have been the result of hetero-fertilization (Sprague, 1932). Plants from the seeds classified as non-shrunken waxy, or shrunken, non-waxy will also be grown. Seeds of these 2 types cannot be accounted for on the basis of hetero-fertilization, and as contamination is rather unlikely, crossing over in the F_1 hybrids is the most probable reason for their occurrence.

The difference between the crossover values reported here and those obtained by Beadle may be due to differences in the Florida teosinte plants used as parents, or they may be due to environmental or genic differences affecting the rate of crossing over (Stadler 1925, 1926). The fact that Beadle used third and fourth generation backcross plants may have had some effect, although the crossover values presented by Emerson and Beadle (1932) for successive backcross generations are uniformly low in all.

If chiasmata formed in meiotic prophase condition diakinesis and equatorial plate association of the members of a bivalent (Darlington 1932) then a test of the assumption that every chiasma represents a crossover can be proposed here. In maize heterozygous for the T8–9a translocation one arm of the pachytene cross-shaped configuration consists of the short arm of the ninth chromosome and approximately 12 genetic map units besides (McClintock 1931). Beadle (1932) finds that crossing over in the *wx*-translocation region is approximately the same in maize-Florida teosinte hybrids as in maize, that is, 12 percent, but in all the short arm

TABLE 7

Interchange configurations in hybrids of T8-9a maize×teosinte.

INVESTIGATOR	TEOSINTE PARENT	MAIZE PARENT	NO. OF PLANTS STUDIED	CHROMOSOMES IN COMPLEX	RING OF 4	CHAIN OF 4	2 "PAIRS"	TRIVALENT AND UNIVAL.	PAIR AND 2 UNIVALENTS	TOTAL	PERCENT RINGS
Beadle	Florida	T8-9a	5	8 and 9	16	62	158	6	2	244	6.46
Arnason	Florida	T8-9a	4	8 and 9	36	17	13	0	1	67	53.7
Arnason	Florida	T1-2a-T8-9a	5	8 and 9	118	61	34	8	11	232	50.9
Beadle	Durango	T8-9a	4	8 and 9	46	183	4	4	0	237	19.0
Arnason	Durango	T1-2a-T8-9a	3	8 and 9	7	12	77	1	8	105	6.7

(52 map units in maize) only 5 percent of crossing over, or less, occurs in the hybrids. One complete arm of the translocation prophase cross is accounted for by the genetic data. A total of 17 percent of crossing over occurs in that whole arm. On BELLING'S theory, with 17 percent of crossing over, there should be one chiasma present in that arm in only 34 percent of the cells. At diakinesis the 66 percent of cells without a chiasma in that arm should show the chromosomes of the T8–9a complex in configurations other than a ring. The exact form taken will depend on the pairing relations of most of the long arm of chromosome 9 and all of chromosome 8 with the teosinte chromosomes in the complex. Thirty-four percent of microsporocytes might be expected to show a chromosome ring configuration at diakinesis if chiasmata are regularly formed in the other 3 arms of the complex. Our counts on diakinesis configurations, reported in the previous section, gave the proportion of cells having a ring of 4 chromosomes at diakinesis as approximately 50 percent. BEADLE (1932) (table 7), however, gives a much lower figure.

On the assumption that often no chiasma is formed in one arm of the eighth chromosome, the low percentage (6.46) of diakinesis rings of 4 chromosomes in the T8–9a maize×Florida teosinte hybrids might appear to be in accord with BELLING'S theory. This is the more plausible when the high frequency of 2 end-to-end pairs is noted. The formation of a chiasma in the yg_2–T region of the ninth chromosome may not, on that account, always insure ring formation. The figures here reported for the first time cannot be explained on that basis. Either too many cells in the doubtful, uncounted class had configurations other than rings, or else the actual number of rings obtained is higher than would be expected on BELLING'S theory. Some of the material had been stored in 70 percent alcohol for some months and was not ideal for critical examination of the T8–9a configuration. Satisfactory preparations, however, were obtained.

No backcross data are available with regard to crossing over in the yg_2–*sh*–*wx*–T region of the ninth maize chromosome in hybrids with Durango teosinte, except those reported by BEADLE (1932). He found complete absence of crossing over in the yg_2–*sh*–*wx* region, but the percentage of crossing over in the *wx*–T region was about the same as in pure maize, namely, 12 percent. The proportion of diakinesis rings reported by him (19 percent) agrees fairly well with what would be expected on BELLING'S theory. In our material, however, the proportion of rings, and chains as well, was much lower, the predominant configuration being 2 end-to-end pairs (plate 1N, a, b, O, c). The high proportion of 2 end-to-end pairs (table 4) suggests that one arm of the eighth chromosome of maize, as well as one of the ninth, often fails to have a chiasma attachment to a Durango teosinte "homolog." Unfortunately, no genetic tests of crossing

TABLE 8

Crossing over in Maize-Florida teosinte hybrids. Backcross data.

MAIZE CHROMOSOME	CONSTITUTION OF HYBRID	NUMBER OF INDIVIDUALS					CROSSOVERS		MAP DISTANCE[1] IN MAIZE
		XY	Xy	xY	xy	TOTAL	NO.	PERCENT	
1	$\frac{br\ f}{+\ +}$	1		2*	3	6	2	33.3	6
	$\frac{f\ bm_2}{+\ +}$	2	1	2*	1	6	3	50.0	64
	$\frac{br\ bm_2}{+\ +}$		1	4	1	6	5	83.3	70
2	$\frac{v_4 gl_2}{+\ +}$	126	114	85	184	509	199	39.1	51
	$\frac{gl_2 lg_1}{+\ +}$	169	42	95	203	509	137	26.9	20
	$\frac{v_4 lg_1}{+\ +}$	140	100	124	145	509	224	44.0	71
3	$\frac{lg_2 d_1}{+\ +}$	12	8	6	15	41	14	34.1	30
7	$\frac{gl_1 v_5}{+\ +}$	72	3	6	95	176	9	5.1	6

[1] Data from EMERSON (14).

* No sign of fine stripes but the classification was not checked by backcrossing to *f* stock.

over in the eighth chromosome of maize in maize-Durango or maize-Florida teosinte hybrids have been reported. Only 2 genes have been located in the eighth chromosome. Adequate tests of crossing over, therefore, are impossible.

CROSSING OVER BETWEEN OTHER CHROMOSOMES IN MAIZE-FLORIDA TEOSINTE HYBRIDS

F_1 plants obtained by crossing linkage tester stocks of maize with Florida teosinte were backcrossed to the maize lines. Genetic tests involving regions of chromosomes 1, 2, 3 and 7 have been made. The results are presented in table 8.

It is established that crossing over occurs between maize and Florida teosinte chromosomes in the regions which were under observation. Where the numbers are large enough to be indicative the crossover values appear to be not very different from those found in pure maize.

Genetic tests by EMERSON and BEADLE (1932) showed that crossing over in segments of chromosomes 2, 5 and 10 in maize-Florida teosinte hybrids was about as frequent as in maize. The short arm of chromosome 9 is the only chromosome segment in which crossing over has been shown to be markedly reduced in these hybrids.

DISCUSSION

The observations on microsporocytes of hybrids between Florida teosinte and strains of maize carrying known translocations has supplied more precise information regarding the degree to which pairing takes place between particular chromosomes of maize and the corresponding chromosomes of teosinte than can be had by observation on microsporocytes of ordinary maize by teosinte hybrids. If the chromosome ring configurations seen in diakinesis stages in plants heterozygous for a translocation owe their formation to previous chiasma formation in each arm of the pachytene cross-shaped structure, then the high proportion of rings seen in hybrids of T1–2a, T1–2aT1–6a, T1–7a with Florida teosinte provides proof that chiasmata are regularly formed at or near both ends of the first, second, sixth and seventh chromosomes. This may be used as evidence that very considerable portions of these maize chromosomes have their structural counterparts in chromosomes of Florida teosinte. Pairing of chromatids at pachytene stages has been shown by various workers to be determined by homology of the pairing segments. Especially instructive are the cross-shaped configurations seen at pachytene stages in microsporocytes of maize heterozygous for reciprocal translocation. These configurations are interpretable on the grounds that chromatids lie in close association where they are homologous; that where homology ends, pairing likewise ends (MCCLINTOCK 1930; COOPER and BRINK 1931). BURNHAM (1931) and MCCLINTOCK (1932), however, reported close association of short non-homologous maize chromosome segments. If pachytene pairing of parts of non-homologous chromatids occurs, and chiasmata are not formed, diakinesis association of chromosomes should be a more reliable indication of correspondence than earlier prophase figures. Should chiasmata occur in the paired regions, translocation, reduplication and deficiency would be expected. The effects would presumably be reflected in gametophyte sterility or aberrant genetic ratios. Intimate association of non-homologous chromosome segments combined with chiasma formation must be rare in maize since the resulting unequal crossing over would be readily detected.

Parts of the fourth, fifth, eighth and ninth chromosomes of maize appear to conjugate with parts of corresponding Florida teosinte chromosomes. The extent of correspondence of the fifth and eighth chromosomes with Florida teosinte "homologs" has not been fully determined but is suspected of being incomplete. The short arm of the ninth maize chromosome almost certainly does not correspond with a part of the teosinte "homolog."

Tests of crossing over in maize-Florida teosinte hybrids have been made for segments of 7 chromosomes by EMERSON and BEADLE (1932) and the writer. In 6 of the chromosomes crossing over appears similar in amount to that in maize. The genetic as well as the cytological evidence indicates that the chromosomes of maize and Florida teosinte correspond closely but that the correspondence is not complete for all the chromosomes.

The results suggest that gene changes rather than changes in the gross structure of the chromosomes may have been chiefly responsible for the differentiation of maize and annual teosinte.

SUMMARY

1. Pairing of chromosomes 1, 2, 6 and 7 of maize and corresponding Florida teosinte chromosomes appears to be complete in hybrids.

2. Relatively long segments of either the eighth or ninth maize chromosome frequently fail to form chiasmata with teosinte "homologs."

3. Only 5 percent of crossing over occurs in maize-Florida teosinte hybrids in a segment of the ninth maize chromosome which includes 52 map units.

4. Some cytological evidence of possible slight structural difference between chromosome 5 of maize and the Florida teosinte chromosome with which it pairs, at least in part, is presented.

5. Crossing over occurs between genes in maize chromosomes 1, 2, 3 and 7 and Florida teosinte chromosomes. The frequency of crossing over in hybrids was not accurately determined but the few data obtained suggest that the values are not very different from those in pure maize.

6. Conclusive evidence is not yet available as to which maize chromo-

somes are members of the two heteromorphic pairs found in microsporocytes of maize-Florida teosinte hybrids. Evidence that chromosomes 1, 2, 6 and 7 are not involved has been obtained. Chromosome 5 may be a member of one of the unequal pairs, the other is probably chromosome 8, 9 or 10.

7. Pairing of chromosomes 1, 2 and 6 of maize with Durango teosinte chromosomes appears to be complete.

8. Cytological evidence suggests that chiasmata are very rarely formed between relatively long segments of both chromosome 8 and 9 of maize with corresponding Durango teosinte chromosomes.

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A MORPHOLOGICAL COMPARISON OF TRIPLOID AND TETRAPLOID INTERSPECIFIC HYBRIDS IN TRADESCANTIA

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ONE of the fundamental methods of genetic investigation has been to vary the balance between selected elements in the germplasm and compare the resulting combinations. Outstanding examples of the successful application of this method are BRIDGE'S (1925) studies of the balance between the sex chromosome and the autosomes and MULLER'S (1931) classification of mutant factors by a comparison of their effects in disomic and trisomic combinations. The American species of Tradescantia, several of which exist in both diploid and auto-tetraploid races (ANDERSON and WOODSON 1935; ANDERSON and SAX, in press) make it possible to apply the same method to the study of specific differences. The following paper reports the results of crosses between a common tetraploid species, *Tradescantia subaspera* Ker-Gawl var. *typica* ANDERSON and WOODSON (*T. pilosa* Lehm.) and diploid and tetraploid races of *T. canaliculata* Raf. (*T. reflexa* Raf.).

THEORETICAL

This experiment provides us with four forms for comparison, the two species, the triploid hybrid, and the tetraploid hybrid. The diploid and tetraploid races of *T. canaliculata* are morphologically indistinguishable, as is generally the case among these American Tradescantias (ANDERSON and SAX, in press). Using the letter S to represent a genom of *T. subaspera* and the letter C to represent a genom of *T. canaliculata*, we may diagram the first species as SSSS, the latter as CCCC (or CC in the case of the diploid race). The two possible hybrids we can denote as CSS and CCSS, thereby emphasizing the fact that the former has two sets of chromosomes from *T. subaspera* but only one from *T. canaliculata*, whereas in the latter hybrid the two species have each contributed two sets.

Assuming that the differences between the two species are genic, or largely so, what differences may we expect between the two hybrids? Let us first of all examine the consequences to be expected if the species are differentiated by dominant genes, dominants and recessives being distributed about equally to both species. In the case of a dominant contributed by *T. subaspera*, the formulae for the two hybrids will be AAa and AAaa. For a dominant gene the effects of these two combinations should be indistinguishable, or practically so. If the dominant is contributed by *T. canaliculata*, the two hybrid formulae are Bbb and BBbb. In this case we shall ordinarily expect the two combinations to be practically indistinguishable, since if we define a dominant gene as one in which the combination AA has practically the same effect as Aa, we know from past experience that for the majority of such genes Aaa is similar in effect to Aa and hence to AAaa. In the hybrids, therefore, if the genes distinguishing the two species are dominant, or largely so, we may expect CCSS and CSS to be essentially similar. Since, in point of fact, the triploid hybrid CSS is strikingly different from the tetraploid hybrid CCSS we may conclude *that the essential differences between the two species, if genic, are largely without dominance.*

EXPERIMENTAL DATA

While the characters which distinguish the species of Tradescantia are not so precise, nor so unaffected by the environment, as one might wish, the two species chosen for the experiment differ as sharply as any two within the group related to *T. virginiana*. Unfortunately, *T. subaspera* var. *typica* is so far known only as an autotetraploid; were a diploid race to be discovered it would permit comparison of three different intermediate balances with the two pure species. The experiment is furthermore unfortunate in the fact that the only tetraploid *T. canaliculata* in flower at the time the crosses were made was a somewhat exceptional individual with slightly broader leaves and a larger number of nodes than is normal for the species. In both of these characteristics it departed from the normal in the direction of *T. subaspera* var. *typica*, thereby reducing slightly what might have been an even greater difference between the triploid and tetraploid hybrids. The node number of this tetraploid parent was so exceptionally high that this character, which would otherwise have been one of the best for comparison, has been omitted altogether. Both crosses were made with *T. canaliculata* as the female parent. For the triploid, a diploid plant from Austin, Texas, was pollinated from a plant of *T. sub-*

TABLE 1

Comparison of triploid and tetraploid hybrids between T. canaliculata and T. subaspera var. typica

	T. canaliculata	TETRAPLOID HYBRID	TRIPLOID HYBRID	*T. subaspera* VAR. *typica*
	CCCC	*CCSS*	*CSS*	*SSSS*
Internode length	16 cm.	11 cm.	8.5 cm.	3 cm.
Leaf length ÷ width	23	16	10	7
Stomata on upper surface	none	few	several	many
Sepal length	1.1 cm.	0.9 cm.	0.8 cm.	0.7 cm.
Pubescence of pedicel	none	slight	medium	heavy

aspera var. *typica* collected at Marthasville, Missouri. The tetraploid had the same pollen parent, its seed parent being a tetraploid plant of *T. canaliculata* from Hamburg, Missouri.

Detailed comparisons for both hybrids with the parental species are made in table 1 and text-figure 1. The internodes used in the table and the figure were chosen from comparable points on all four plants, being the first internode below the lowest branch of the inflorescence and the leaf subtending this internode. It will be noted from figure 1 that this character is apparently somewhat affected by hybrid vigor. Leaf shape is probably the most reliable of the characters, since it is only slightly affected by hybrid vigor and by environmental influences. It will be seen that the hybrid CCSS is almost exactly intermediate between the two species in its

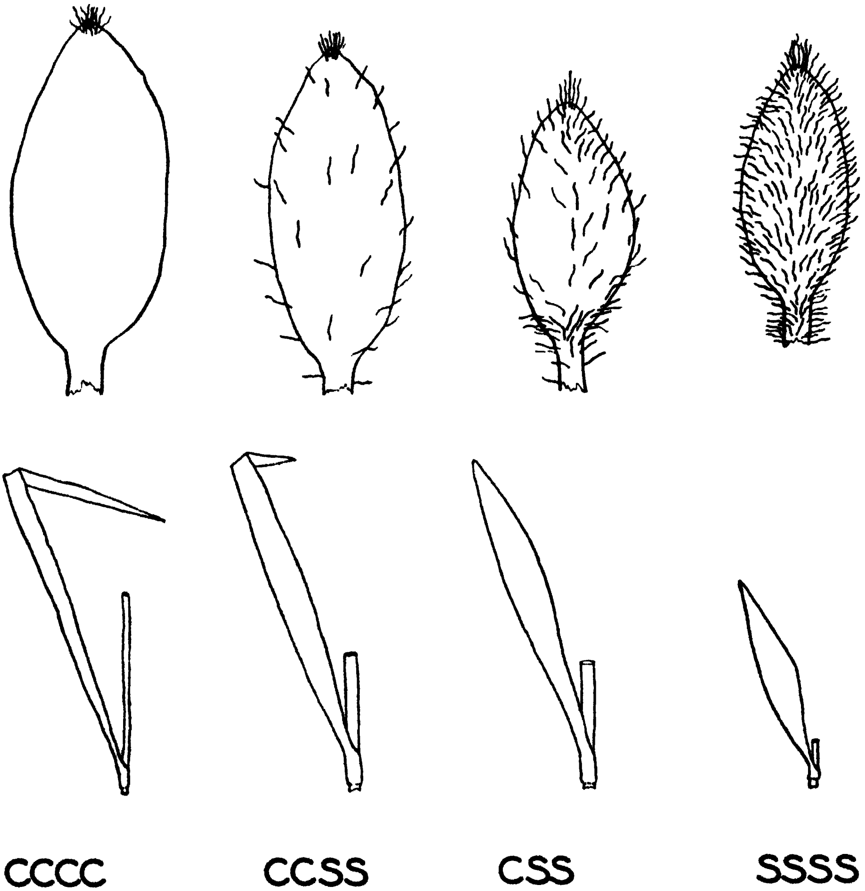

FIGURE 1.—Above: Calyx and pedicels (×10); Below: Leaf and internode (×1.3) of T *canaliculata* (CCCC), *T. subaspera* (SSSS), their triploid (CSS) and tetraploid (CCSS) hybrids. While the calyces have been carefully drawn to scale they are somewhat diagrammatic.

leaf index and that the triploid hybrid CSS is again almost exactly intermediate between it and SSSS.

The relative abundance of stomata on the upper and lower surfaces of the leaf is a character of much taxonomic importance in the genus Tradescantia (ANDERSON and WOODSON 1935). In *T. subaspera* var. *typica* there are virtually no stomata on the upper surface; in *T. canaliculata* they are about equally abundant on both surfaces. The two hybrids CSS and CCSS present two intermediate stages between these end points. Table 1 and figure 1 present a tabular and graphical summary of the five characters most susceptible to precise comparison. In each case the tetraploid hybrid CCSS is intermediate between the two species and the triploid CSS is intermediate between it and SSSS. Other less objective characters presented a similar result; young plants of CCSS, for instance, were in general aspect clearly intermediate between the two parents and were recognized as such by naturalists familiar with the two species. The plants of CSS, however, particularly in their younger stages, presented more the appearance of a puzzling variant of *T. subaspera*, to those unacquainted with the origin of the plants.

SUMMARY AND CONCLUSIONS

As measured by five unrelated characters, tetraploid hybrids between *T. subaspera* var. *typica* and *T. canaliculata* were exactly intermediate between the two species. By the same measure, triploid hybrids to whose germplasm *T. subaspera* var. *typica* had made twice the contribution of *T. canaliculata*, were intermediate between the tetraploid hybrids and *T. subaspera* var. *typica*. Following the argument developed in the theoretical section, it is concluded that in so far as the differences between these two species are genic, they rest largely upon genes with incomplete dominance.

NOTE

It is perhaps worth calling attention to the taxonomic significance of the facts reported in the above paper. Polyploidy introduces a number of complications into the relationships between species. In this case the two races of *T. canaliculata* are morphologically indistinguishable, yet outcrossed to another species, *T. subaspera*, they produce two sets of hybrids which are very dissimilar morphologically, one of which (the triploid) is almost completely sterile, and one of which (the tetraploid) is semi-fertile. The case illustrates one out of many possible ways in which crosses between the same two species can give different results under different conditions.

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STUDIES OF A SIZE CROSS IN MICE

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IT WILL be conceded generally by students of genetics that body size, though clearly subject to heredity, is not inherited in a simple Mendelian way.

When races of unlike body size are crossed, the offspring are, in general, intermediate in body size, both in the first and in later generations of offspring. Most investigators in this field, being convinced that Mendel's law is universally valid, have accepted what is known as the multiple factor interpretation of this and other similar cases of intermediate or blending inheritance. On this hypothesis the genetic basis of differences in body size consists exclusively of mutated genes borne in the chromosomes. It is certain that such genes exist. Specific evidence for their existence will be described in this paper. But we are not convinced that no cell structures other than chromosomes are concerned in the determination of body size for the following reasons.

Some years since, a study was made of the inheritance of body size in a cross between races of rabbits, one of which was about four times as large as the other. The blending character of the inheritance indicated that, on a multiple factor interpretation, differential genes influencing body size must be so numerous and widely distributed as to be found in practically all chromosome pairs. An attempt was accordingly made, by crossing a large race in which four chromosomes carried dominant color genes with a small race carrying the corresponding recessive genes, to ascertain whether these four chromosome pairs bore any of the hypothetical genes influencing body size. The result was wholly negative. No difference in size could be detected between segregates which bore one or all of the dominant genes received from the large race parent and those which bore recessive genes derived from the small race parent. Nevertheless by embryological studies, CASTLE and GREGORY were able to show that the eggs of large-race and small-race rabbits develop at a different rate. Though the eggs are of the same size, that of the large-race rabbit undergoes cleavage more rapidly and thus produces a larger embryo. The more rapid rate of growth persists after birth and even until adult size is attained. Moreover, sperm as well as egg is influential in determining the developmental rate of the zygote. It seems, accordingly, that developmental rate is itself either gene controlled or controlled by some other feature of the organization of both sperm and egg.

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About the time that this conclusion had been reached in our rabbit studies, GREEN made a cross between two species (or subspecies) of mice (*Mus bactrianus* and *Mus musculus*), the former of which is only about half as large as the latter. He applied the same criterion which CASTLE had used in the rabbit size cross for possible linkage between color genes and size genes, but with better success. He reported association in F_2 and backcross populations of larger size with the recessive color genes brown, dilution, and non-agouti, which came from the larger parent in the original cross. He inferred correctly that this implied the location in the three chromosome pairs under consideration of genes influencing body size. Later, when larger numbers had been studied, he concluded that the differences reported were of significant magnitude in one only of the three cases—that of the chromosome carrying the gene for brown. On this alone he based the argument for a multiple factor interpretation of body-size inheritance, supporting it later by evidence thought to indicate crossing over between the brown gene and one or more size genes located in the same chromosome.

We shall show that GREEN was undoubtedly right in assigning an influence making for greater body-size to both the brown chromosome and the dilute chromosome. The case for the non-agouti chromosome is doubtful. But we shall show, also, that the plus influence on body-size exerted by the brown chromosome and the dilute chromosome is due not, probably, to some hypothetical size gene linked with the color gene, but to the physiological action of that gene itself.

Our original plan was to verify, if possible, GREEN's findings by repeating his experiment with certain minor modifications. In this we had the cordial coöperation of Dr. GREEN, who supplied us with animals of the same inbred stocks which he had used in his experiment—namely, *M. bactrianus* and LITTLE's well known and long inbred dilute brown strain, both from the JACKSON MEMORIAL LABORATORY. We owe hearty thanks, in this connection, to Director C. C. LITTLE, and Drs. GREEN and MURRAY.

In the first cross which we made, on which we shall report in this paper, we used as mothers, not LITTLE's dilute brown race but a derivative of it established by GATES, in which pink-eye and short-ear had been added to the assemblage of recessive characters. This race accordingly was homozygous for the five recessive characters, pink-eye, dilution, short-ear, brown, and non-agouti. It had been closely inbred in brother-sister matings for several generations. Two of the five recessive genes, namely, dilution and short-ear, are borne in the same chromosome and presumably very close together, since the occurrence of crossovers is only about one in 1000. Accordingly, four chromosomes are in this race tagged with recessives, namely, (1) brown, (2) dilution and short-ear, (3) pink-eye, and (4) non-agouti. For brevity, we shall refer to this race as the s.e. race. As the other parent race we used not wild *M. bactrianus*, as GREEN had done, but a supposedly domestic derivative of it, the long inbred strain of black-and-white Japanese waltzing mouse used by Gates (1926). This carries three independent recessive characters, piebald, waltzing, and non-agouti.

In weight 17 adult males of the s.e. race range from 22 to 33 grams, average 26.2. Gates (1926) reports the average weight of adult Japanese waltzing males to be 17.6 grams. The weight of five adult F_1 males ranges from 21.0 to 29.8 grams, average 25.4. These fragmentary records indicate (and the general impression which one gets when handling the animals coincides with it) that the F_1 animals are nearly, though not quite, as large as the larger parental race. In vigor, productiveness, and longevity, however, the hybrids are far superior to either parent race.

The F_1 animals were crossed reciprocally with LITTLE's dilute brown race in order that segregation in size might be observed in relation to brown and dilution only without complication from pink-eye and short-ear which would continue to be recessive in whatever backcross animals received them from the F_1 parent. For the "d br" race, having been deliberately inbred, chiefly in brother-sister matings, since 1909, according to GREEN, should be completely homozygous for all genetic factors (except for possible new mutation), and it is free from pink-eye and short-ear, though homozygous for dilution and brown.

The backcross animals accordingly fall, as regards color, into four classes, (1) black, (2) dilute black (blue), (3) brown, and (4) dilute brown.

GATES (1926) thought that there was a tendency in a cross similar to this for the chromosomes to emerge from the cross in the same associations in which they entered it, so that in F_2 or in backcrosses a higher percentage was obtained of character combinations identical with those found in the parents than one would expect from chance alone. But GREEN did not observe any such tendency toward an association between chromosomes in the cross which he made between *M. bactrianus* (the supposed ancestor of the waltzing mouse) and the "d br" race of LITTLE. Further, a reëxamination of the data recorded by GATES raises a question as to the significance of the statistical deviations from expectation which he reported. It has seemed desirable, therefore, to reopen the question in connection with this investigation.

If GATES' association principle were valid for this cross, we should expect the parental combinations *intense black* and *dilute brown* to exceed in frequency the recombinations *dilute black* and *intense brown*. In the cross between F_1 females and "d br" males a population of 1001 animals recorded at weaning time (age 3 to 4 weeks) has been tabulated from the records without selection. They consist of (1) black intense, 235; (2) black dilute, 268; (3) brown intense, 249; and (4) brown dilute, 249. The sum of the parental combinations (black intense and brown dilute) is 484, which is less than the sum of the recombinations (black dilute and brown intense), which equals 517. The probable error (expected chance deviation from equality in a population of this size) is 10.6. The observed deviation is 16.5, which is less than twice the P. E. and so not significant. It is also contrary in character (being minus rather than plus) to a deviation which might be due to persistent association of the chromosomes. The conclusion is warranted that in this cross, as in so many others which have been studied, the segregation of each chromosome pair occurs quite independently of other chromosome pairs, and continued association or dissociation of genes borne in different chromosomes is quite a matter of chance. We may therefore dismiss as groundless a criticism by Castle of Green's interpretation of his results based on the association principle outlined by Gates.

From the cross, F_1 ♀ × "d br" ♂, 1236 mice have been reared, weighed monthly or oftener between the ages of 4 and 6 months, and then chloroformed and measured as to body length and tail length by Sumner's method, keeping the body under a uniform tension of 20 grams. In tables 1 and 2 the animals are classified as to sex, color, and maximum weight at or prior to six months of age. In tables 3 and 4 they are similarly classified as regards body length at six months of age.

The order of increasing size is the same for weight and for body length in both sexes. Blacks are smallest, then come blues, next browns, and last dilute browns. The only exception is found in body weight of dilute brown females, which are no heavier than intense browns, although as regards body length they are decidedly longer than intense browns. Body length is, as suggested by Green, a more reliable indicator of general body size than weight, for certain individuals in both the F_1 and the backcross populations have a tendency to become very fat after they have reached the age of 4 or 5 months, so that their weights displace the average upward. Body length, on the other hand, is apparently not affected by accumulation of fat, though it is clear that only individuals of large size become excessively fat. Body length is also a linear dimension, whereas increase in body weight depends upon increase in all correlated dimensions and thus produces greater variation. This is shown in the higher coefficients of variability for weight than for body length. For females the C. V. in weight is 10.8–12.0 and in body length 3.4–3.6; for males the C. V. in weight is 9.8–11.1, and in body length 2.9–3.8.

If we compare all black pigmented individuals (whether intense or dilute) with all brown pigmented ones of the same sex (whether intense

TABLE 1

Variation in weight of females of four different color classes from matings, ♀ F_1 × ♂ *d br* (*Little*).

COLOR	LOWER LIMITS OF WEIGHT CLASSES IN GRAMS																		NO.	MEAN	S.D.	C.V.
	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32				
Black	2	1	3	15	16	28	35	23	13	11	6	5	1	0	1				160	21.46±.12	2.33±.08	10.8
Blue		2	2	11	17	24	21	35	23	16	11	9	0	0	4	0	1	1	177	22.32±.13	2.69±.09	12.0
Brown			4	6	13	22	30	20	21	11	17	11	2	5	2				164	22.61±.13	2.62±.09	11.5
Dilute Brown			4	5	10	28	24	28	21	12	12	10	4	2	1	2	1		164	22.61±.14	2.67±.10	11.8
All Blacks	Black and Blue combined																		337	21.91±.08	2.55±.05	Dif. means,
All Browns	Brown and Dilute Brown combined																		328	22.61±.12	2.65±.07	.70±.14
All Intense	Black and Brown combined																		324	22.04±.09	2.52±.06	Dif. means,
All Dilute	Blue and Dilute Brown combined																		341	22.45±.10	2.67±.07	.41±.13

TABLE 2

Variation in weight of males of four different color classes from matings, ♀ F_1 × ♂d br (Little).

COLOR	LOWER LIMITS OF WEIGHT CLASSES IN GRAMS																								NO.	MEAN	S.D.	C.V.
	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41				
Black	1	0	0	0	3	2	13	13	12	18	21	13	19	6	6	3	3	1	2						136	28.24±.16	2.77±.10	9.8
Blue		1	2	1	3	5	10	8	17	17	13	18	14	9	11	7	1	0	3	0	2	1	1		144	28.64±.17	3.04±.12	10.6
Brown					2	5	3	14	10	15	23	22	17	6	7	3	7	3	3	0	2				142	29.09±.18	3.24±.13	11.1
Dilute Brown						2	1	4	14	18	19	23	24	13	7	6	6	4	2	2	2	0	1	1	149	30 01±.17	3.18±.12	10.5
All Blacks	Black and Blue combined																								280	28.34±.13	3.40±.09	Dif. means,
All Browns	Brown and Dilute Brown combined																								291	29.56±.12	3.23±.09	1.22±.17
All Intense	Black and Brown Combined																								278	28.67±.12	3.16±.09	Dif. means,
All Dilute	Blue and Dilute Brown combined																								293	29.34±.13	3.52±.09	.67±.17

TABLE 3

Variation in body length in mm. of females of four color classes from matings, ♀ F_1 × ♂d br (Little).

COLOR	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	104	NO.	MEAN	S.D.	C.V.
Black		2	1	0	5	4	8	10	19	28	22	17	10	7	8	12	2	1	1	1			158	90.88±.17	3.22±.12	3.54
Blue	1	0	0	0	3	4	5	10	12	15	24	23	23	15	18	4	9	6	2	1	1		176	92.13±.17	3.26±.12	3.53
Brown				1	1	2	5	6	13	14	11	21	16	25	23	5	12	3	2	1	1		162	92.68±.17	3.21±.12	3.46
Dilute Brown		1	0	1	1	3	1	8	10	12	11	21	14	19	21	15	10	4	4	2	0	1	159	93.00±.18	3.40± 12	3.65
All Blacks	Black and Blue combined																						334	91.51±.12	3.32±.08	Dif. means,
All Browns	Brown and Dilute Brown combined																						321	92.84±.12	3.26±.08	1.33±.17
All Intense	Black and Brown combined																						320	91.79±.12	2.72±.08	Dif. means,
All Dilute	Blue and Dilute Blue combined																						335	92.55±.12	3.36±.08	.74±.17

TABLE 4

Variation in body-length in mm. of males of four color classes from matings, ♀ F_1 × ♂ d br (Little).

COLOR	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	NO.	MEAN	S.D.	C.V.
Black	1	0	0	1	0	1	1	3	2	7	6	14	9	20	13	15	15	14	6	4	2	1				135	95.52 ± .20	3.47 ± .14	3.63
Blue		2	0	0	1	1	2	2	2	5	7	9	10	12	18	20	22	12	7	4	1	2	0	2		141	95.91 ± .20	3.68 ± .14	3.83
Brown							3	1	1	1	7	4	8	15	14	23	19	21	13	4	2	1	2	1	1	141	96.96 ± .17	3 09 ± .12	3.18
Dilute Brown							1	0	1	1	0	3	5	9	13	16	26	23	14	12	11	6	2	1	2	146	98 46 ± .16	2 93 ± .11	2.97
All Blacks	Black and Blue combined																									276	95.77 ± .14	3.50 ± .10	Dif. means,
All Browns	Brown and Dilute Brown combined																									287	97.73 ± .10	3.11 ± .08	1.96 ± .17
All Intense	Black and Brown combined																									276	96.26 ± .14	3 38 ± .10	Dif. means,
All Dilute	Blue and Dilute Brown combined																									287	97.21 ± .14	3.57 ± .10	.95 ± .17

or dilute), we find the latter invariably longer-bodied and heavier in both sexes. For females, browns are 1.4 percent longer-bodied and 3.2 percent heavier; for males, browns are 2.04 percent longer-bodied and 4.3 percent heavier. See table 5.

If we compare the intense pigmented individuals (whether black or brown) with dilute pigmented (whether black or brown) of the same sex, we find the latter invariably larger and heavier. The difference is less in the case of dilution than it was in the case of brown, but still significant statistically in every case. See table 5. For females, the dilutes are 0.82 percent longer-bodied and 1.9 percent heavier; for males, the corresponding values are 0.98 percent and 2.3 percent.

TABLE 5

Comparison of females with males as regards body length and weight in different color groups.

AV. BODY LENGTH	FEMALES	MALES	RATIO
All Blacks	91.55	95.77	100:104.6
All Browns	92.84	97.73	100:105 2
Ratio, Black:Brown	100:101.40	100:102.04	Mean = 104.9
All Intense	91.79	96.26	100:104 8
All Dilute	92.55	97.21	100:105.0
Ratio, Intense:Dilute	100:100.82	100:100.98	Mean = 104.9
Av. Weight			
All Blacks	21.91	28.34	100:129
All Browns	22.61	29.56	100:131
Ratio, Black:Brown	100:103.2	100:104.3	Mean = 130
All Intense	22.04	28.67	100:130
All Dilute	22.45	29.34	100:130
Ratio, Intense:Dilute	100:101.9	100:102.3	Mean = 130

The correlation between body-length and weight is high, being 0.65 ± .01 in the case of females and 0.66 ± .01 in the case of males. See tables 6 and 7. This supports the conclusion which one of us has based on a long series of investigations indicating that the genetic agencies which affect size are chiefly general rather than local in their action.

An interesting relation between the size characters of females and males is shown in table 5. Males are consistently about 5 percent longer-bodied than females of the same color group, but in weight they are about 30 percent heavier, the first difference being based on a linear measurement, the second on a mass or three-dimensional evaluation.

The differences between color groups are also greater relatively, as well as absolutely, in the case of males when compared with females. Brown makes an increase in body length (over black) of 1.40 percent in the case of females but of 2.04 percent in the case of males. Brown also increases weight by 3.2 percent in the case of females, but by 4.3 percent in the case of males.

Dilution consistently has a lesser effect on size than brown, but its effect is regularly more pronounced in the case of males than of females. Thus dilution increases body length by .82 percent in females, but by .98 percent in males. It increases weight by 1.9 percent in females, but by 2.3 percent in males. The male mouse is a larger animal than the female. It grows beyond the stage at which growth is normally arrested in females, and in the final stages of growth the differential effect of genes borne in the chromosomes carrying brown and dilution is more strongly in evidence than before.

It remains to consider the question whether the differential effect on growth exercised by the brown and dilution chromosomes is due to the genes for brown and dilution respectively or to some gene or genes linked with them. FELDMAN (1935) has recently published data which indicate that in races of mice, whether of large or of small body size, browns in mixed litters are regularly larger than their black sibs.

In the cross described in this paper, we introduced from the *musculus* parent into the hybrids two closely linked recessive characters borne in the same chromosome, namely, dilution and short-ear. We have shown that in the backcross to "d br" males the individuals which are homozygous for dilution are larger than those which are heterozygous (the blacks and browns). Dilution (or something linked with it) must be regarded as an influence increasing size when homozygous. Short-ear does not reappear in this backcross, though present as a recessive in substantially all dilute individuals, since it would be transmitted to all individuals to which the F_1 parent transmitted dilution, unless a crossover occurred between short-ear and dilution.

But in a different backcross, to be described more fully in a subsequent paper, short-ear does make its appearance, and we are thus able to estimate its influence on size. When our F_1 females are backcrossed to the parental *musculus* race (pink-eyed short-eared dilute brown), the same four classes of offspring result as in the backcross already described, namely, black, blue, brown, and dilute brown; but in this case blues and dilute browns are short-eared. They are also regularly inferior in size to the blacks and browns respectively, which are long-eared. In other words, the tendency of homozygous dilution to *increase* body size, whether associated with black or with brown, is more than offset by a tendency of short-ear, when homozygous, to *decrease* body size.

But dilution and short-ear are closely linked with each other, with crossovers occurring about once in a thousand times. Any hypothetical size gene closely linked with one should also be closely linked with the other. Yet homozygous dilution increases size, and homozygous short-ear

TABLE 6

Correlation between body length and weight in 656 backcross females. $r = .65 \pm .01$.

	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	
15		1								1															2
16	1				1	1																			3
17		1	1	2	2			6																	12
18					5	6	3	7	6	4	3		1	1			1								37
19			1		1	3	9	4	12	5	6	8		3		1									53
20						1	3	9	14	25	10	16	7	5	7	2									99
21					1	1	3	3	9	15	18	23	13	9	8	3	2	1							109
22								2	8	9	20	16	15	16	10	6	3								105
23						1	1	1	2	5	3	10	11	12	16	9	5	3							79
24									2	3	4	4	6	6	13	3	4	3	1						49
25								1			3	3	4	5	7	8	7	4	2	2					46
26									1	1	1	1	5	7	6	3	5		2	2	1				35
27										1		1		1	1		1		2						7
28															1	1	2	2	1						7
29												2			1		2	1		1	1				8
30																	1							1	2
31														1				1	1						3
	1	2	2	2	10	13	19	33	54	69	68	84	62	66	70	36	33	15	9	5	2			1	656

TABLE 7

Correlation between body length and weight in 534 backcross males. r = .66 ± 01.

	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	
19					1																				1
20	2																								2
21							1																		1
22				1		1	1		2				1	1											7
23						4	1	2	1	2	1	1	1		1										14
24					1	1	1		4	2	6	5	3	2		2									25
25						1			1	8	7	4	5	2	4	2	1	1							36
26							1	2	2	3	5		9	6	4	6	1			1					40
27							1		3	1	7	7	11	12	14	4	6	1							67
28											4	2	10	9	14	15	11	6	1		1				73
29												3	7	11	12	17	11	9	1	4					75
30								1		2		1	6	10	14	9	13	8	4	2		1	1		72
31								1				1			2	9	7	5	3	3					31
32													2	2	2	6	5	1	6		4		1	1	30
33														1	2	4	2	2	4	1		1			17
34															1	2	6	1		1	2	1		1	15
35																	3	2	1	1					7
36									1					1		2	2	2	1	1					10
37																						1			1
38															1	1				1			1	1	5
39															1										1
40																1					1	1			3
41																			1						1
	2			1	2	7	6	6	13	19	30	22	55	57	72	80	68	38	22	15	8	5	3	3	534

decreases size, even in the presence of dilution. It is difficult to avoid the conclusion that these genes are, by virtue of their own physiological action, genes influencing body size. For if the influence on size were due to a third gene linked with short-ear and dilution, it should become operative when *either* short-ear or dilution become homozygous; but we find that size increases when dilution becomes homozygous but decreases when short-ear becomes homozygous, their action being qualitatively contrary.

SUMMARY

1. A cross was made between a pink-eyed dilute brown short-eared race of house mouse and an inbred strain of black-and-white Japanese waltzing mouse. Adult males of the parent races weighed, on the average, 26.2 and 17.6 grams, respectively. The F_1 hybrids were uniform black in color, and males, when adult, weighed about 25.4 grams.

2. A backcross was made between F_1 hybrid females and males of LITTLE's dilute brown race. The progeny fall into four color classes, black, blue, brown, and dilute brown, with no statistically significant differences between their respective frequencies. A backcross population of 665 females and 571 males was raised to the age of six months, each animal being weighed at monthly intervals and its body-length measured at six months of age.

3. In body-size, whether estimated by maximum weight or by body length, brown animals are larger than blacks, and dilute animals are larger than intense ones, the difference being greater in the case of brown than of dilution, but clearly significant in both.

4. Males are larger than females and show a greater influence on size of the brown and the dilute characters than females do.

5. Reasons are given for regarding the superior size of brown and dilute animals as due to the physiological action of the genes for those characters rather than of specific size genes borne in the same chromosomes.

6. The coefficient of correlation between adult weight and body length is, in the case of females, .65 ± .01, and of males, .66 ± .01.

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STUDIES IN HUMAN INHERITANCE XIII

A TABLE TO DETERMINE THE EXPECTED PROPORTION OF FEMALES SHOWING A SEX-INFLUENCED CHARACTER CORRESPONDING TO ANY GIVEN PROPORTION OF MALES SHOWING THE CHARACTER

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IN A PREVIOUS paper (Snyder, L. H. and Yingling, H., 1935) the gene frequency method was applied to sex-influenced factors, and a formula for testing the applicability of the hypothesis of sex-influenced factors to human data was derived. Since any character dependent upon sex-influenced factors will usually be relatively frequent in males, but relatively rare in females, another method of attack presents itself. Knowing the proportion of males showing a character suspected of being due to a sex-influenced factor, what proportion of females may be expected to show the character, assuming random mating? As a practical example, if 40% of males are bald, what proportion of females may be expected to be bald, assuming that baldness is due to a sex-influenced factor and that random mating occurs in regard to this character?

Assume a pair of allelomorphs *B* and *b*, such that *B* is dominant in males, but recessive in females. Let p = frequency of *B*, and q = frequency of *b*. Then $p+q=1$. Here p and q may be separately derived, as follows:

$$\frac{p^2}{2}=\text{proportion of } BB\,♀♀ \text{ in general population,}$$

$$\frac{q^2}{2}=\text{proportion of } bb\,♂♂ \text{ in general population.}$$

Since the proportion of *BB* ♀♀ in the general population is equal to half of their proportion among females alone, and the proportion of *bb* ♂♂ in the general population is equal to half of their proportion among males alone, we may write as follows:

Let $\overline{♀B}$ = proportion among females of females who show the character represented by *B*, let $\overline{♂B}$ = proportion among males of males who show the character represented by *B*, and let $\overline{♂b}$ = proportion among males of males who show the character represented by *b*. Then

$$\frac{p^2}{2}=\frac{\overline{♀B}}{2}$$

$$p=\sqrt{\overline{♀B}} \tag{1}$$

$$\frac{q^2}{2}=\frac{\overline{♂b}}{2}$$

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$$q=\sqrt{\overline{\text{♂}b}}. \tag{2}$$

So that

$$\sqrt{\overline{\text{♀}B}}+\sqrt{\overline{\text{♂}b}}=1. \tag{3}$$

From equation (3) it is possible to derive a value of $\overline{\text{♀}B}$ in terms of $\overline{\text{♂}B}$.

$$\begin{aligned}\sqrt{\overline{\text{♀}B}}+\sqrt{\overline{\text{♂}b}}&=1\\ \sqrt{\overline{\text{♀}B}}&=1-\sqrt{\overline{\text{♂}b}}=1-\sqrt{1-\overline{\text{♂}B}}\\ \overline{\text{♀}B}&=\left(1-\sqrt{1-\overline{\text{♂}B}}\right)^2.\end{aligned} \tag{4}$$

From equation (4) a table may be constructed, giving, for any proportion of males showing a dominant sex-influenced character, the corresponding proportion of females who may be expected to show the character. Employing the maximum likelihood method of R. A. FISHER (1930), the probable error formula for equation (4) may be derived as follows:

The distribution of dominant and recessive individuals in a sample of N males follows the terms of the expansion of the binomial, $[(p^2+2pq)+q^2]^N$. More exactly, the chance P of getting n dominants and N−n recessives is

$$P=K(p^2+2pq)^n(q^2)^{N-n}=K(1-q^2)^n(q^2)^{N-n} \tag{5}$$

where

$$K=\frac{N!}{n!(N-n)!}\,.$$

The value to be estimated is p^2, so that setting

$$\theta=p^2$$

we obtain

$$q=1-\sqrt{\theta}.$$

Putting this value of q in equation (5), we get

$$P=K(2\sqrt{\theta}-\theta)^n(1-\sqrt{\theta})^{2N-2n}. \tag{6}$$

Taking natural logarithms,

$$\begin{aligned}L=\log P&=\log K+n\log(2\sqrt{\theta}-\theta)+(2N-2n)\log(1-\sqrt{\theta})\\ \frac{\partial L}{\partial\theta}&=\frac{n(1-\sqrt{\theta})}{\theta(2-\sqrt{\theta})}-\frac{N-n}{\sqrt{\theta}-\theta}\\ \frac{\partial^2 L}{\partial\theta^2}&=\frac{(N-n)(1-2\sqrt{\theta})}{2\sqrt{\theta}(\sqrt{\theta}-\theta)^2}-\frac{n}{2\theta\sqrt{\theta}(2-\sqrt{\theta})^2}-\frac{n(1-\sqrt{\theta})}{\theta^2(2-\sqrt{\theta})}\,.\end{aligned} \tag{7}$$

Substituting $\theta=p^2$ and $n=Np(2-p)$, we have

$$\frac{\partial^2 L}{\partial(p^2)^2}=-\frac{N}{p^3(2-p)}\,. \tag{8}$$

The variance of p^2,

$$\sigma^2 p^2 = -\frac{1}{\frac{\partial^2 L}{\partial (p^2)^2}} = \frac{p^3(2-p)}{N}\,. \tag{9}$$

Substituting $p = 1 - \sqrt{1 - \overline{\text{♂}B}}$, we get

$$\text{P.E. } p^2 = \pm .6745\left(1 - \sqrt{1 - \overline{\text{♂}B}}\right)\sqrt{\frac{\overline{\text{♂}B}}{N}}\,. \tag{10}$$

The complete equation involved is thus

$$\overline{\text{♀}B} = \left(1 - \sqrt{1 - \overline{\text{♂}B}}\right)^2 \pm .6745\left(1 - \sqrt{1 - \overline{\text{♂}B}}\right)\sqrt{\frac{\overline{\text{♂}B}}{N}}\,. \tag{11}$$

From table 1, for any proportion of males showing a dominant sex-influenced character, the proportion of females who may be expected to show the character may be directly read.

TABLE 1

Table of values of $\overline{\text{♀}B} = \left(1 - \sqrt{1 - \overline{\text{♂}B}}\right)^2$

The values of $\overline{\text{♂}B}$ *to two decimal places are given in the left-hand column; the third decimal place for each value is given in the top row Thus for* $\overline{\text{♂}B} = .310$*, the proportion of* $\overline{\text{♀}B}$ *is .0287; for* $\overline{\text{♂}B} = .316$ *it is .0299.*

$\overline{\text{♂}B}$	.000	.001	.002	.003	.004	.005	.006	.007	.008	.009
.000	.0000	.0000	.0000	.0000	.0000	.0000	.0000	.0000	.0000	.0000
.010	.0000	.0000	.0000	.0000	.0000	.0001	.0001	.0001	.0001	.0001
.020	.0001	.0001	.0001	.0001	.0001	.0002	.0002	.0002	.0002	.0002
.030	.0002	.0002	.0003	.0003	.0003	.0003	.0003	.0003	.0004	.0004
.040	.0004	.0004	.0005	.0005	.0005	.0005	.0005	.0006	.0006	.0006
.050	.0006	.0007	.0007	.0007	.0007	.0008	.0008	.0008	.0009	.0009
.060	.0009	.0010	.0010	.0010	.0011	.0011	.0011	.0012	.0012	.0012
.070	.0013	.0013	.0013	.0014	.0014	.0015	.0015	.0015	.0016	.0016
.080	.0017	.0017	.0018	.0018	.0018	.0019	.0019	.0020	.0020	.0021
.090	.0021	.0022	.0022	.0023	.0023	.0024	.0024	.0025	.0025	.0026
.100	.0026	.0027	.0027	.0028	.0029	.0029	.0030	.0030	.0031	.0031
.110	.0032	.0033	.0033	.0034	.0034	.0035	.0036	.0036	.0037	.0038
.120	.0038	.0039	.0040	.0040	.0041	.0042	.0042	.0043	.0044	.0045
.130	.0045	.0046	.0047	.0047	.0048	.0049	.0050	.0050	.0051	.0052
.140	.0053	.0054	.0054	.0055	.0056	.0057	.0058	.0058	.0059	.0060
.150	.0061	.0062	.0063	.0063	.0064	.0065	.0066	.0067	.0068	.0069
.160	.0070	.0071	.0072	.0072	.0073	.0074	.0075	.0076	.0077	.0078
.170	.0079	.0080	.0081	.0082	.0083	.0084	.0085	.0086	.0087	.0088
.180	.0089	.0090	.0091	.0092	.0093	.0095	.0096	.0097	.0098	.0099
.190	.0100	.0101	.0102	.0103	.0104	.0106	.0107	.0108	.0109	.0110

TABLE 1. (*Continued*)

Table of Values of $\overline{♀B} = \left(1-\sqrt{1-\overline{♂B}}\right)^2$

$\overline{♂B}$	.000	.001	.002	.003	.004	.005	.006	.007	.008	.009
.200	.0111	.0113	.0114	.0115	.0116	.0117	.0119	.0120	.0121	.0122
.210	.0124	.0125	.0126	.0127	.0129	.0130	.0131	.0133	.0134	.0135
.220	.0136	.0138	.0139	.0140	.0142	.0143	.0145	.0146	.0147	.0149
.230	.0150	.0151	.0153	.0154	.0156	.0157	.0159	.0160	.0161	.0163
.240	.0164	.0166	.0167	.0169	.0170	.0172	.0173	.0175	.0176	.0178
.250	.0179	.0181	.0183	.0184	.0186	.0187	.0189	.0191	.0192	.0194
.260	.0195	.0197	.0199	.0200	.0202	.0204	.0205	.0207	.0209	.0210
.270	.0212	.0214	.0215	.0217	.0219	.0221	.0222	.0224	.0226	.0228
.280	.0229	.0231	.0233	.0235	.0237	.0238	.0240	.0242	.0244	.0246
.290	.0248	.0250	.0251	.0253	.0255	.0257	.0259	.0261	.0263	.0265
.300	.0267	.0269	.0271	.0273	.0275	.0277	.0279	.0281	.0283	.0285
.310	.0287	.0289	.0291	.0293	.0295	.0297	.0299	.0301	.0303	.0305
.320	.0308	.0310	.0312	.0314	.0316	.0318	.0320	.0323	.0325	.0327
.330	.0329	.0332	.0334	.0336	.0338	.0340	.0343	.0345	.0347	.0350
.340	.0352	.0354	.0357	.0359	.0361	.0364	.0366	.0368	.0371	.0373
.350	.0375	.0378	.0380	.0383	.0385	.0388	.0390	.0393	.0395	.0398
.360	.0400	.0403	.0405	.0408	.0410	.0413	.0415	.0418	.0420	.0423
.370	.0425	.0428	.0431	.0433	.0436	.0439	.0441	.0444	.0447	.0449
.380	.0452	.0455	.0457	.0460	.0463	.0466	.0468	.0471	.0474	.0476
.390	.0479	.0482	.0485	.0488	.0491	.0494	.0497	.0499	.0502	.0505
.400	.0508	.0511	.0514	.0517	.0520	.0523	.0526	.0529	.0532	.0535
.410	.0538	.0541	.0544	.0547	.0550	.0553	.0556	.0559	.0562	.0565
.420	.0568	.0572	.0575	.0578	.0581	.0584	.0587	.0591	.0594	.0597
.430	.0600	.0604	.0607	.0610	.0613	.0617	.0620	.0623	.0627	.0630
.440	.0633	.0637	.0640	.0644	.0647	.0650	.0654	.0657	.0661	.0664
.450	.0668	.0671	.0675	.0678	.0682	.0685	.0689	.0692	.0696	.0699
.460	.0703	.0707	.0710	.0714	.0718	.0721	.0725	.0729	.0732	.0736
.470	.0740	.0744	.0747	.0751	.0755	.0758	.0762	.0766	.0770	.0774
.480	.0778	.0782	.0786	.0789	.0793	.0797	.0801	.0805	.0809	.0813
.490	.0817	.0821	.0825	.0829	.0832	.0837	.0841	.0846	.0850	.0854
.500	.0858	.0862	.0866	.0870	.0875	.0879	.0883	.0887	.0891	.0896
.510	.0900	.0904	.0909	.0913	.0917	.0922	.0926	.0930	.0935	.0939
.520	.0944	.0948	.0952	.0957	.0961	.0966	.0970	.0975	.0980	.0984
.530	.0989	.0993	.0998	.1003	.1007	.1012	.1016	.1021	.1026	.1031
.540	.1035	.1040	.1045	.1050	.1054	.1059	.1064	.1069	.1074	.1079
.550	.1084	.1089	.1093	.1098	.1103	.1108	.1113	.1118	.1123	.1128
.560	.1134	.1139	.1144	.1149	.1154	.1159	.1164	.1169	.1175	.1180
.570	.1185	.1190	.1196	.1201	.1206	.1212	.1217	.1222	.1228	.1233
.580	.1239	.1244	.1249	.1255	.1260	.1266	.1271	.1277	.1283	.1288
.590	.1294	.1299	.1305	.1311	.1316	.1322	.1328	.1334	.1339	.1345

TABLE 1. (*Continued*)

Table of Values of $\overline{♀B} = \left(1-\sqrt{1-\overline{♂B}}\right)^2$

$\overline{♂B}$	.000	.001	.002	.003	.004	.005	.006	.007	.008	.009
.600	.1351	.1357	.1363	.1368	.1374	.1380	.1386	.1392	.1398	.1404
.610	.1410	.1416	.1422	.1428	.1434	.1440	.1446	.1453	.1459	.1465
.620	.1471	.1477	.1484	.1490	.1496	.1502	.1509	.1515	.1521	.1528
.630	.1534	.1541	.1547	.1554	.1560	.1567	.1574	.1580	.1587	.1593
.640	.1600	.1607	.1613	.1620	.1627	.1634	.1640	.1647	.1654	.1661
.650	.1668	.1675	.1682	.1689	.1696	.1703	.1710	.1717	.1724	.1731
.660	.1738	.1745	.1752	.1760	.1767	.1774	.1781	.1789	.1796	.1803
.670	.1811	.1818	.1826	.1833	.1841	.1848	.1856	.1863	.1871	.1879
.680	.1886	.1894	.1902	.1909	.1917	.1925	.1933	.1941	.1949	.1957
.690	.1964	.1972	.1980	.1988	.1997	.2005	.2013	.2021	.2029	.2037
.700	.2046	.2054	.2062	.2070	.2079	.2087	.2096	.2104	.2113	.2121
.710	.2130	.2138	.2147	.2156	.2164	.2173	.2182	.2190	.2199	.2208
720	.2217	.2226	2235	.2244	.2253	.2262	.2271	.2280	.2289	.2298
.730	.2308	.2317	.2326	.2336	.2345	.2354	.2364	.2373	.2383	.2392
.740	.2402	.2412	.2421	.2431	.2441	.2450	.2460	.2470	.2480	.2490
.750	.2500	.2510	.2520	.2530	.2540	.2551	.2561	.2571	.2581	.2592
.760	.2602	.2612	.2623	.2633	.2644	.2655	.2665	.2676	.2687	.2698
.770	.2708	.2719	.2730	.2741	.2752	.2763	.2774	.2785	.2797	.2808
.780	.2819	.2831	.2842	.2853	.2865	.2876	.2888	.2900	.2911	.2923
.790	.2935	.2947	.2959	.2971	.2983	.2995	.3007	.3019	.3031	.3043
.800	.3056	.3068	.3081	.3093	.3106	.3118	.3131	.3144	.3156	.3169
.810	.3182	.3195	.3208	.3221	.3234	.3248	.3261	.3274	.3288	.3301
.820	.3315	.3328	.3342	.3356	.3370	.3383	.3397	.3411	.3425	.3440
.830	.3454	.3468	.3482	.3497	.3511	.3526	.3540	.3555	.3570	.3585
.840	.3600	.3615	.3630	.3645	.3661	.3676	.3691	.3707	.3723	.3738
.850	.3754	.3770	.3786	.3802	.3818	.3834	.3851	.3867	.3883	.3900
.860	.3917	.3933	.3950	.3967	.3984	.4002	.4019	.4036	.4054	.4071
.870	.4089	.4107	.4125	.4143	.4161	.4179	.4197	.4216	.4234	.4253
.880	.4272	.4291	.4310	.4329	.4348	.4368	.4387	.4407	.4427	.4447
.890	.4467	.4487	.4507	.4528	.4548	.4569	.4590	.4611	.4633	.4654
.900	.4675	.4697	.4719	.4741	.4763	.4786	.4808	.4831	.4854	.4877
.910	.4900	.4923	.4947	.4971	.4995	.5019	.5043	.5068	.5093	.5118
.920	.5143	.5169	.5194	.5220	.5246	.5273	.5299	.5326	.5353	.5381
.930	.5408	.5436	.5465	.5493	.5522	.5551	.5580	.5610	.5640	.5670
.940	.5701	.5732	.5763	.5795	.5827	.5860	.5892	.5926	.5959	.5993
.950	.6028	.6063	.6098	.6134	.6170	.6207	.6245	.6283	.6321	.6360
.960	.6400	.6440	.6481	.6523	.6565	.6608	.6652	.6697	.6742	.6789
.970	.6836	.6884	.6933	.6984	.7035	.7088	.7142	.7197	.7254	.7312
.980	.7372	.7433	.7497	.7562	.7630	.7701	.7774	.7850	.7929	.8012
.990	.8100	.8193	.8291	.8397	.8511	.8636	.8775	.8935	.9126	.9378

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THE TEMPERATURE-EFFECTIVE PERIODS AND THE GROWTH CURVES FOR LENGTH AND AREA OF THE VESTIGIAL WINGS OF DROSOPHILA MELANOGASTER

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INTRODUCTION

ROBERTS (1918) first reported the increase in size of the wings of the vestigial mutant of *D. melanogaster* when reared at high temperatures and showed that the effect was more pronounced in the males than in the females. STANLEY (1928) stated that "the length of vestigial wing varies directly with the temperature, but not in direct proportion." I have shown (1930a) that there is a critical temperature for the increase in the length of the vestigial wings and that the marked change occurs in the females at a temperature 1° higher than in the males, a response showing sexual dimorphism. The curves for total development at one temperature of STANLEY (1931) and mine (1930a) were similar over the temperature range examined (17° to 31°). In preliminary reports (1930b, 1932, 1933) I have indicated that the temperature-effective period for increasing the size of the vestigial wings at 30°, 31°, 32°, and 33° C. is during the larval period commencing at approximately 60 hours and extending for various intervals with different maximum rates depending upon the sex of the individuals and the temperature experienced by the larvae. STANLEY (1931) found entirely different temperature-effective periods at 17° and 27° but in his recent paper (1935) he reports intervals at 30° and 31° easily harmonized with the periods reported here.

STOCKS AND METHODS

An inbred stock of vestigial winged flies should be homozygous for practically all the genes concerned in wing development and consequently the variability due to recombinations of modifying genes would be reduced to a minimum. Single pair brother-sister matings were made and selection was practiced in each generation, the sib matings being made from the bottle showing the best yield and with the flies showing the largest vestigial wings. The selection practiced should accelerate the approach to homozygosity.

The food used in the experiments was the customary banana-agar jell. The bananas used throughout the experiments were uniformly overripe (the skins dark brown), but not decayed, and a 1 percent agar-agar medium was used. The food was poured to a depth of 25 mm. in 1×4 inch vials and yeasted 24 hours before the parents entered the vials. The same batch of food was used at both temperatures and for all time intervals in any one trial.

In the final experiments the same parents produced the eggs for the trials at 30° and 32°, 31° and 32°, and 31° and 33° C.; repeated trials being alternated between these temperature combinations. Thus comparable data should be produced for these four temperatures. One-hour egg-laying periods at 25° with eight pairs of flies per vial were used for the experiments at 30°, 31°, and 32°. The number of eggs deposited in each vial was below the limits of "the crowding effect" on the wings. These vials remained in the 25° incubator for 24 hours. At the end of this period practically all the eggs had hatched Harnly (1929). Preliminary tests had shown no temperature effect on the wings during the first 48 hours of development. The vials were then placed at two of the experimental temperatures. Eggs from the same parents were placed at all time intervals at both temperatures, thus distributing any residual genetic variability uniformly throughout the experiment. Beginning at 48 hours of total development and every 4 hours thereafter sets of vials were returned to the 25° incubators for the completion of development and the emergence of the adult flies.

A temperature of 33° was nearly 2° above the lethal point for the complete development and emergence of the flies of this vestigial stock. A procedure was developed which would carry a fair number to puparium formation at 33°. A 3-hour egg-laying interval was used and the first 48 hours of development took place at 25°. The larvae in vials transferred at this time from 25° to 33° entered the temperature-effective period some twelve or more hours later. Beginning at 72 hours of total development and every 12 hours thereafter sets of vials were transferred from 33° to 25° for the completion of development and emergence of the adult flies. The larvae had great difficulty in forming puparia at 33°. Most of them moved up on the walls of the vials and crawled around on the surface of the glass for several hours until death resulted apparently from excessive drying. To overcome this hazard the larvae were removed from the walls of the vials with a moistened camel's hair brush every 2 to 4 hours and returned to the surface of the food during the puparium formation period at 33°. With this procedure many of them succeeded in forming puparia and, if placed at a lower temperature presumably before the time of pupation proper, succeeded in completing their development and emerging as adult flies. No emerged flies have been obtained from total development at either 32° or 33° with this stock. Total and partial development was tried repeatedly at 34°, 35°, and 36° without much success.

The accuracy of the incubators used was ± 0.05° C., the temperatures being determined through the double glass door with burette readers from standardized thermometers graduated to 0.1° C. The incubators used in our laboratory for temperature work are all small units with only one shelf and an electric fan maintains a forced circulation of the air within the incubator. The thermostats are grids of glass tubing containing toluol and are closed by a mercury column having a movement in the capillary arm of an inch and a half per degree centigrade. The thermostat carries a very small current thus preventing sparking and fouling of the mercury surface and it controls the polarity on the grid of a radio tube. This in turn operates a telegraph relay carrying on its rocker arm a tube in which the heating circuit is made and broken in mercury, thus removing the common difficulty of frozen contact points in the heating circuit and the resultant death of the cultures. The lag is negligible and the heating circuit is made and broken on an average of once in 60 to 90 seconds. The air accuracy of these incubators is ±0.05° C. but tests with thermocouples placed in the surface of the food in which the larvae are feeding show no perceptible fluctuation between the on and off position in the temperature of the food surrounding the larvae. This control mechanism will maintain a constant temperature for months without any adjustment. The 25° incubators were kept in a cold room (10° C.) which had a variation of less than 1° and the 30°, 31°, 32°, and 33° incubators were kept in a room where the temperature varied from 20° to 22° C.

The right wings of the emerged flies were removed under a binocular microscope, mounted in 95 percent alcohol under a cover slip, projected with a compound microscope and a 500 watt lamp, and drawn. The linear magnification was 115. The lengths were determined by projecting a Leitz 2 mm slide ruled to 0.01 mm onto the drawings. The areas were determined with a Keuffel and Esser compensating polar planimeter no. 4242. The mean values for each time interval were determined from the lengths and areas of an average of fifty males and fifty females except the last few points at each temperature. Due to the high death rate for long exposures these points are the mean value of twenty-five to forty surviving individuals. Six trials were made at each temperature.

TEMPERATURE-EFFECTIVE PERIODS

A. Preliminary tests

The preliminary tests on the temperature-effective period were reported in London (HARNLY 1930 b). They were made on the twelfth generation of brother-sister matings with transfers from 31° and 32° to 29° and reciprocal transfers from 29° to 31° C. Since the preliminary experiments had shown that the temperature-effective period began late in the third day of development it was not necessary in the final tests to make transfers from the high temperatures to 25° before the first 48 hours of development had been completed. This allowed a check interval at the high temperatures of over 12 hours before the beginning of the critical period.

B. Periods and growth curves for length and area

The final experiments were performed at 30°, 31°, and 32° with transfers at 4-hour intervals to 25° C. and at 33° with 12-hour transfer periods. These experiments extended from the thirty-first through the fiftieth generation of the sib matings. In figures 1, 2, and 3 are plotted the mean length of the male and female vestigial wings for the total developmental time intervals at which the individuals were transferred from the higher temperature to the 25° incubators for the completion of development and emergence.

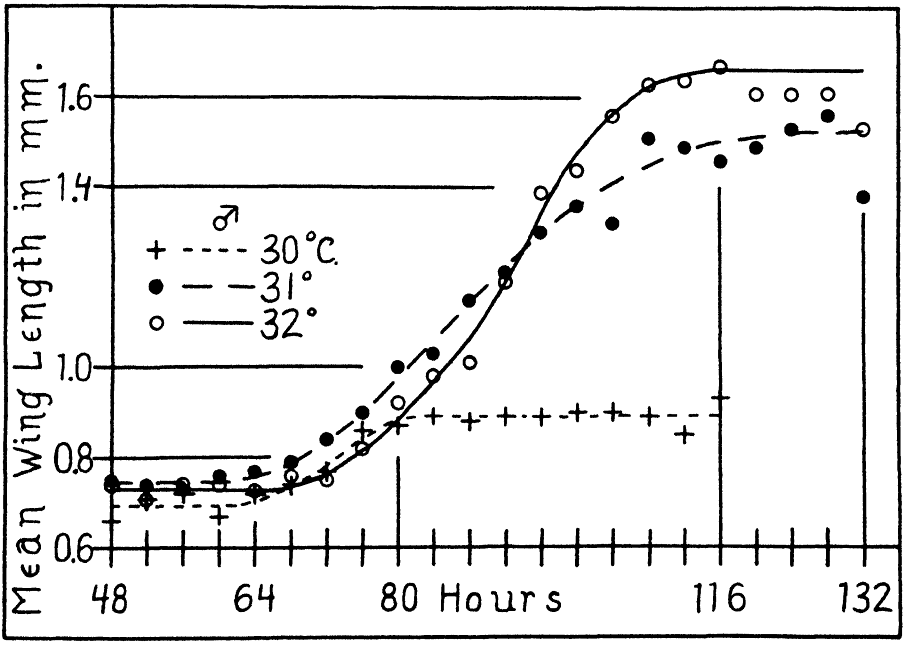

FIGURE 1

From the curves (smoothed by eye) it is evident that the temperature-effective period for wing length began for the males at 30° between 60 and 64 hours and ended between 80 and 84 hours of total development, a duration of some 20 hours, and terminating 27 hours before the mean time of puparium formation (111 hours). At 31° the rise began in the curve for the males at the same time as at 30° (that is, 64 hours) but instead of leveling off at 84 hours it continued upward as a sigmoid curve approaching its asymtote at 112 hours, the mean time of puparium formation for this stock at 31°. A rise of 1° had affected neither the length of the egg-larval period nor the inception of the temperature-effective period but had changed the termination time; its duration being prolonged from 20 hours to 48 hours, an increase of 140 percent, accompanied by a 67 percent increase in wing length. Thirty-one degrees was close to the lethal point and 32° was above it for total development. Furthermore the larval period was prolonged something over 24 hours at 32° and the initiation of the temperature-effective period was retarded approximately 4 hours (68 hours) in comparison with 30° and 31° (64 hours). The duration of the effective period was again 48 hours (to 116 hours), though puparium formation did not come until much later. The wings of the males at 32° were longer than those developed at 31°; this increase of 1° had not affected the duration of the temperature-effective period but did result in a higher maximum rate of wing development. The major change in the vestigial wings of the males at 32° was in the area. Due to the high mortality during the latter part of the developmental period at 33° only sufficient data was obtained to establish the five points between 72 hours and 120 hours. The linear equation

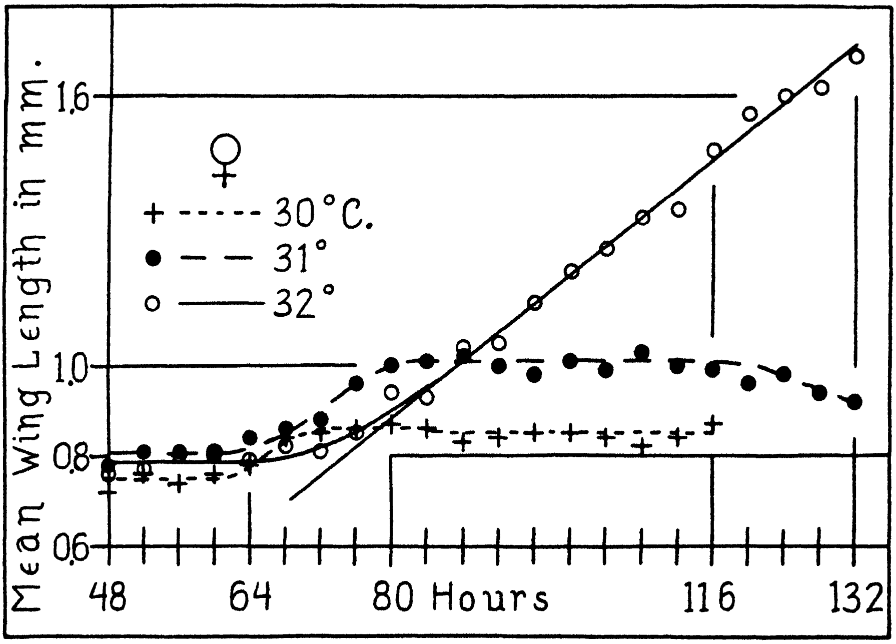

FIGURE 2

$$y = 0.01592x - 0.357$$

appears to describe these five points.

The female period for wing length at 30° began between 60 and 64 hours and ended between 68 and 72 hours of total development (puparium formation 107 hours), an effective period of some 8 hours. Commencing at the same time at 31° the effective period was lengthened from 8 to 20 hours, the curve leveling off between 80 and 84 hours. The termination point for the females at 31° was not associated with puparium formation which came some 30 hours later (114 hours). The growth curve for the females at 32° started approximately 4 hours later than at 31° but instead of leveling off around 84 hours it proceeded upward apparently as a straight line through the rest of the time interval examined. The linear equation

$$y = 0.01592x - 0.391$$

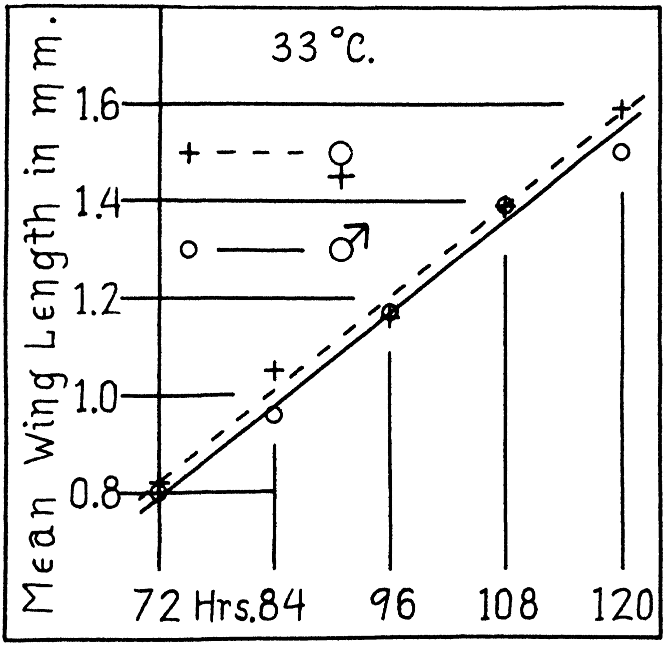

FIGURE 3

appears to describe all the points determined during this temperature-effective period with the exception of the first few points. It is obvious from the data and an inspection of the figures that the rises of 1° from 30° to 31° and from 31° to 32° though they did not significantly affect the inception of the temperature-effective period in the females did markedly affect its termination, the periods at 30°, 31°, and 32° being respectively 8, 20 and some undetermined period more than 70 hours in duration. The five points at 33° for which sufficient data was obtained appear to fit the equation

$$y = 0.01592x - 0.326.$$

The equations for the females at 32° and the males and females at 33° differ only in the intercept, the slope being the same for all three.

The close fit of the straight line drawn for the females at 32° to all the points determined experimentally, except the first few points in the temperature-effective period, raises the question as to whether or not the beginning of this period was sharply defined in time. From the curves in figures 1 and 2, either the temperature-effective period began at the same moment in time for all the individuals and the rate increased during the first few hours, or individuals all of the same chronological age entered this critical period of ontogeny at different times and each of the first two or three points represented more and more individuals in the effective period. Even the most superficial examination of the drawings for either sex at any one of these temperatures demonstrates conclusively that the latter alternative was correct for both the beginning and end of the period. The temperature-effective period as used here denotes that interval in time from the entrance of the first few larvae into the critical period until the last have passed beyond it. The value is that of the whole population. In work of this type with *D. melanogaster* it is impossible to determine this value for a single individual since only one measurement in time can be made on it. The results presented here make possible a new technique by which more accurate values will probably be obtained in subsequent work with other alleles at this locus. Attention has been called to this problem in recent work on the scute and Bar loci (CHILD 1935, MARGOLIS 1935).

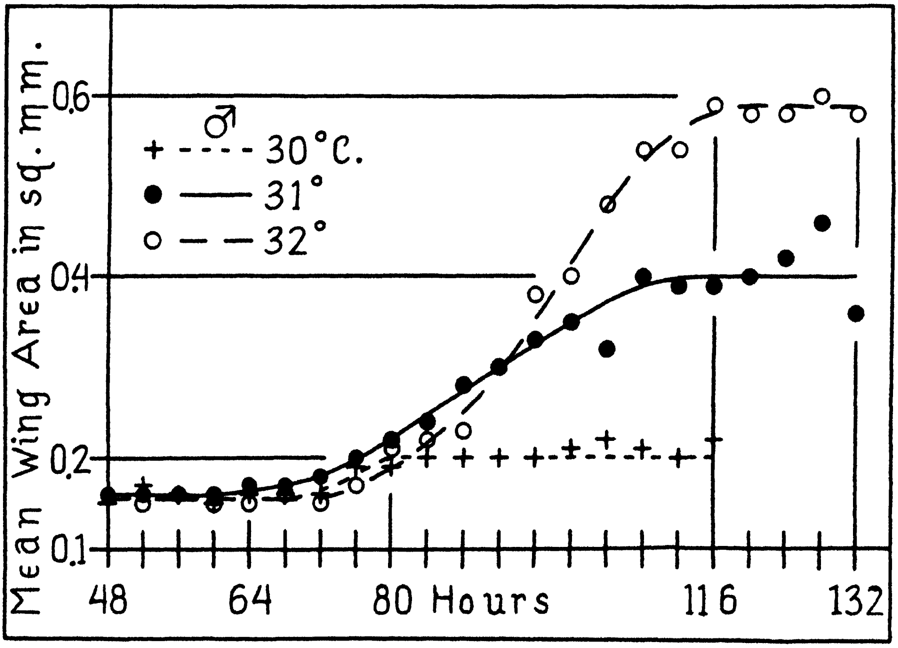

FIGURE 4

The wing areas are plotted against time of transfer in figures 4, 5, and 6. When one considers the data for the males at 30°, 31°, and 32° in figures 1 and 4 it is obvious that there are only minor differences in the curves and time intervals for the length and for the area of the wings. The effective period for wing area began slightly later than that for wing length. It would seem that the beginning of the period involved a slight increase in length at the expense of the width with no resultant change in area. The termination times for both length and area seem to have coincided at each temperature. For area as for length the duration of the temperature-effective period was prolonged markedly with a rise of 1° from 30° to 31° and there was a pronounced increase in the maximum rate with no significant change in the duration at 32°. The major change in wing form from 31° to 32° was in the width and area of the wing accompanied by only a minor increase in wing length. The curve for area at 33° was very definitely not linear and the wing forms were similar to those at 32° for the males.

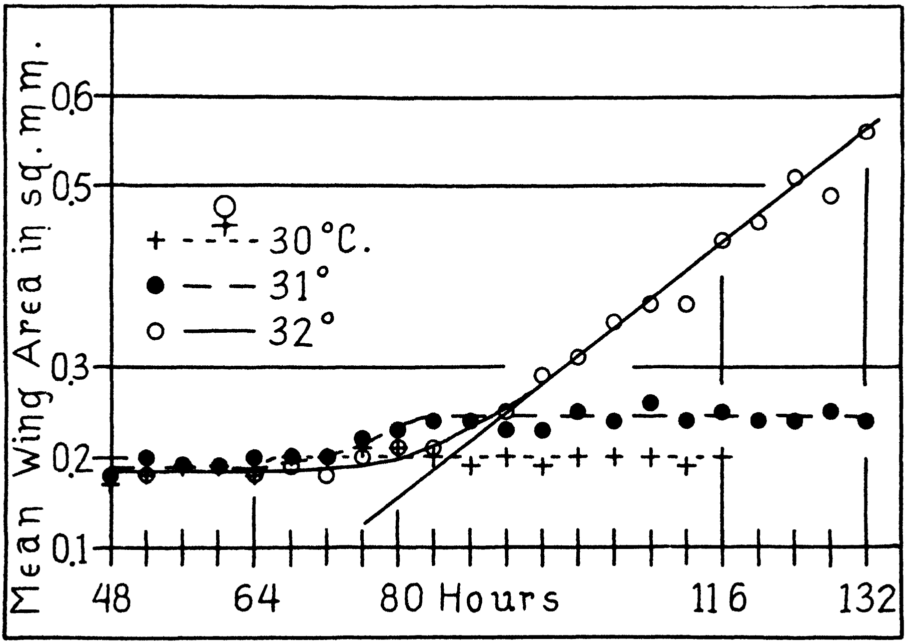

Figure 5

The changes in wing length and wing area for the females were likewise very similar. The beginning of the temperature-effective period seemed to come a little later for wing area than for wing length but they both ended apparently at the same time for each temperature. The rises of 1° from 30° to 31° and from 31° to 32° each lengthened the effective period significantly. The five points determined for wing area of the females at 33° were not linear.

The sexual dimorphism previously reported at high temperatures (ROBERTS 1918; HARNLY 1930a, b; STANLEY 1928, 1931) was obviously due to differences between the males and the females both in rates of "wing formation" and durations of the effective period at each temperature. At 30° the length of the temperature-effective period of the males was several times that of the females. The effective period for the females at 31° was 20 hours (male duration at 30°) and the curve was practically identical in time with that for the males at 30°, but with a higher value throughout. However, the male duration had changed at 31° from 20 hours to 48 hours, continuing the sexual dimorphism at this temperature. A rise of 1° did not modify the inception of the temperature-effective period but had retarded its termination differently for the two sexes. The same length and area was attained in the wings of the males and the females at 32° but it is very evident from the curves that the growth processes were markedly different and the effective period was probably considerably longer for the females than for the males. If they could have been carried for a longer period at 32° the curves indicate that the wings of the females might have been larger than those of the males at this temperature. In the trials at 32° sets of vials were carried for transfer periods considerably beyond 132 hours since it was known that puparium formation came later there. Unfortunately 132 hours was practically the time toleration limit and only a few individuals were obtained beyond that point. These occasional survivors had still larger wings and, though they do not prove it, agree with the assumption that if development could go through to completion of the temperature-effective period for the females at 32°, the two sexes would still show a difference in wing size, but at this point the wings of the females would be larger than those of the males; a complete reversal of the sexual dimorphism to that found in the wild type wings.

In the light of this assumption the curves for the males and the females at 33° are of interest. The mean values for the wings of the females were larger than those of the males at the five points examined between 72 and 120 hours (figures 3, 6), a reversal of the position of the growth curves of the two sexes for vestigial. It has been shown that the wild type wing and many other structures vary inversely with temperature (LUTZ 1913, ALPATOV and PEARL 1929, ALPATOV 1930b, EIGENBRODT 1930, IMAI 1933, STANLEY 1935). STANLEY (1935) has found that the wings of homozygous long-winged (wild type) females were consistently and markedly longer than those of the males for each transfer interval during the temperature-effective periods at 17°, 27°, and 30° C. and the longer the developmental period passed at the higher temperatures, the smaller the wings. Both variation with temperature and with time and the relative sizes of the wings of the two sexes were reversed in homozygous vestigial flies as opposed to the normal allele. But at 33°, though the wing size still increased with time its relative size for the males and females had been changed toward the wild type condition. This indicates that there had been a change in the developmental processes producing in these curves of the male and female vestigial genotypes at 33° a partial simulation of the normal long-wing genotype growth curves. However it was only a partial simulation since wing size for the vestigial flies still varied directly with the length of time the larvae were exposed to 33°. Furthermore there was never any close approach to the normal phenotype in either sex genotypically homozygous for vestigial. As I have pointed out before (HARNLY 1932), the mutant gene vestigial seems to determine the minimum and maximum size of the wings, the potential size being determined by the vestigial gene together with other genes affecting the wings and the exact expression is determined by the gene complex acting in a specific environment during development.

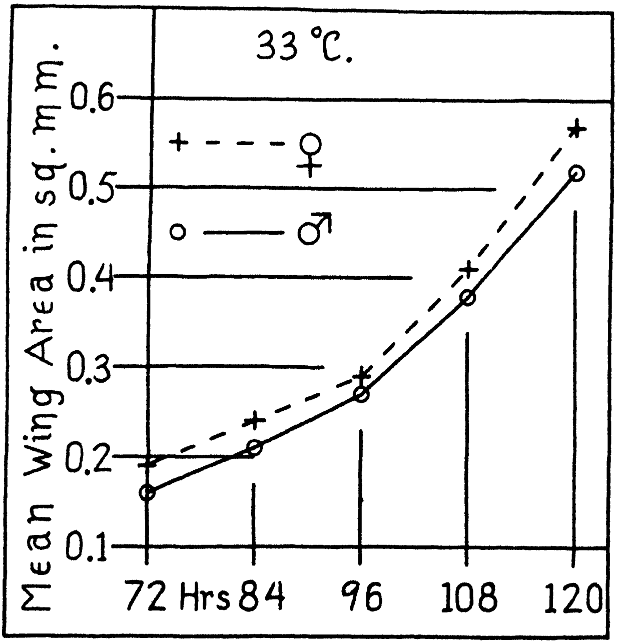

FIGURE 6

From the data presented above it was evident that the temperature-effective period ended long before the time of puparium formation for the males and the females at 30° and for the females at 31° though here the two events seemed to coincide in the males. At 32° the effective period terminated in the males long before puparium formation but this relationship could not be determined for the females due to the lethal effect of exposures beyond 132 hours at this temperature. An experiment was performed to determine whether or not the effective period had ended in the males by the time puparia were formed at 31°.

C. Termination test

Eggs from the same parents were obtained by repeated 1-hour egg-laying periods and allowed to hatch at 25° (24 hours development). The vials were then transferred to 31° for the larval period. With the beginning of puparium formation hourly examinations were made and the newly formed puparia isolated on agar-agar slants in vials. Each hourly set of puparia (prepupae) from a given vial were divided into three groups, consequently any differences due to the number of hours passed in the egg-larval period would be uniformly distributed through the test. At each hourly isolation the first group of puparia were immediately placed at 25° for further development and emergence; the second group of isolated puparia passed an additional 6 hours at 31° and were then transferred to the 25° incubators; and the third group spent 12 hours more at 31° (by which time pupation proper had occurred in all of them) before they were placed in the 25° incubator for further development and emergence. There was no further increase in size due to exposure to 31° beyond the hour of puparium formation.

TEMPERATURE AND GENERAL RATE OF DEVELOPMENT

Any interpretation of the results depends on whether these high temperatures affected proportionately or disproportionately the duration of the effective period and the duration of the general developmental periods, especially the duration of the egg-larval period. The following experiments were performed to answer this question.

A. Methods

The usual procedure for the exact timing of development was followed (LI 1927, HARNLY 1929). Single pair matings of the inbred vestigial flies were used. The eggs were deposited on blotting-paper slips which were then placed on end in 1×4 inch vials containing food 25 mm deep. All the eggs were laid during 1-hour periods at 25° C. At the end of this interval the slips were removed and so distributed that approximately equal numbers of eggs from each female were placed at 25°, 29°, 30°, 31°, and 32°. All subsequent development took place at the temperatures indicated. Hourly examinations were made during the hatching period and at the time of puparium formation. Each puparium was placed on a 2 percent agar-agar slant in a separate vial. The time of emergence and sex of each adult was determined by hourly examinations of these isolated puparia during the emergence period.

B. Effects on the rate of development

The mean duration of the embryonic period (hours required to hatch the eggs) was 21.7 hours at 25°. This was practically the same value found previously for vestigial flies and the frequency curve conformed to the earlier one showing no signs of a bimodal distribution (HARNLY 1929). The frequency distribution curves for hatching at the higher temperatures were also simple sharp unimodal curves. The maximum effect occurred between 25° and 29°, and over the range of 29° to 32° there was no significant change in the duration of this developmental period (19.4 to 19 hours). The major effect of temperature on the length of the embryonic period occurred below the critical temperatures which were found to increase the size of the vestigial wings (30° ♂ ♂, 31° ♀ ♀).

The durations of the egg-larval periods (hours from egg-laying to puparium formation) for the males and the females are stated in table 1. An increase of 4° (25° to 29°) reduced the egg-larval period 17 hours (14 percent) for the males and 10 hours (8.6 percent) for the females, these changes appearing in a temperature range below the critical points for the wings of the males and the females. A rise from 29° through the critical point for the vestigial wings of the males to 30° produced an increase of 6.7 hours in the egg-larval period of the males, but no change occurred in the duration of the egg-larval period for the females. Conversely a rise in temperature through the critical point for the wings of the females from 30° to 31° had only a very slight effect on the egg-larval period of the males but lengthened this period 7.2 hours for the females. The lethal point for complete development of the vestigial winged flies was practically reached at 31°, very few adults emerging, and had been passed at 32°. Consequently the egg-larval period was determined for those larvae which formed puparia at 32° and as no adult flies emerged the sexes were not separated.

TABLE 1

Duration of developmental periods in hours, 4 trials.

°C.	EGG-LARVAL		EGG-LARVAL-PUPAL	
	MEAN± P.E.	σ	MEAN± P.E.	σ
25♂	121.35±1.37	10.37	229.81±1.48	11.18
♀	117.37±1.09	8.38	219.42±1.39	10.50
29♂	104.14±0.82	6.53	192.69±0.91	7.26
♀	106.78±0.87	8.26	192.63±0.96	9.13
30♂	110.86±1.61	12.83	195.79±1.10	8.75
♀	106.83±0.82	5.95	189.88±0.83	6.02
31♂	112.22±1.03	4.59	199.78±1.42	6.32
♀	114.00±0.97	3.22	197.80±1.63	5.42
32	140.07±1.64	18.55		

From the egg-larval-pupal periods and the differences between them and the egg-larval periods in table 1 there was evidently little change in this period above 29° through the critical temperatures for the males and the females (30° and 31°). The pupal period may be ignored in this discussion since there was no differential effect of temperature through the critical range, and since it has been shown above that the temperature-effective period fell entirely within the larval period of development.

The difference of 6.7 hours in the egg-larval periods between the males at 29° and 30° represented an increase of 6.4 percent in this period and was 3.72 times the probable error which is just statistically significant and is based on only a very limited number of surviving individuals. In comparison with this small and questionable increase of the egg-larval period from 29° to 30° a pronounced lengthening of the wings by some 35 percent occurred with the same rise of 1° in temperature (HARNLY 1930a). Furthermore at 30° the temperature-effective period was 20 hours and bore no obvious relation to either the small questionable increase of the egg-larval period or to the periods found by STANLEY (1931) at 17° and 27°. As has been shown above with identical egg-larval periods at 30° and 31° there was an increase of 140 percent in the duration of the temperature-effective period and the wings were lengthened 67 percent at 31°. The same situation was found throughout for the females. In the light of these facts and all the data it seems probable that the effects of temperature above 29° on the duration of the major periods of development (embryonic, egg-larval, and egg-larval-pupal) offer no explanation of either the critical temperatures or the sexual dimorphism. Temperature through its effect on the duration of the critical period for wing development and the rate during that period affects not only the length and area of the wings produced, but also through the same processes the wing form or pattern. The result is not simply more wing of the same kind but a definite ordered progression toward the normal type of wing.

GROWTH PATTERN OF WING FORM

Genotypically homozygous vestigial flies show an interesting succession of phenotypes when reared at 29°, 30°, 31°, and 32°. Typical vestigial wings are produced at 29° and all temperatures below this point. The wings of the females reared at 30° are still typical but those of their brothers are much larger and in appearance markedly over-sized vestigial wings. The wings of the females at 31° have increased greatly in size as the male wings did at 30° and in form are the same type of over-sized or giant vestigial wing. But the wings typical for their brothers at 31° are no longer vestigial but the equivalents of the mutants strap to antlered in phenotype. Finally at 32° these genotypically vestigial males and females have wings that are phenotypically the equivalent of exaggerated notched, or the heterozygote Wild/Carved mutants with parts of the lateral margins missing in many cases. The changes of this genotype with temperature simulating phenotypically other alleles of vestigial reared at 25°, together with the complete progression of the genotype dimorphos vestigial through the vestigial allelic series from the phenotype vestigial at 16° to strap, antlered, Snipped, notched, Carved, vestigial-Beaded, nick, nicked, and finally wild or normal at 32°, and the fact that the homozygous genotype dimorphos vestigial through the temperature range of 16° to 32° duplicated all of the phenotypes produced in MOHR's (1932) combinations of mutants at the vestigial locus at 25° C., led to the propounding of a theory of wing pattern in development in time and the hypothesis that mutations at the vestigial locus involved changes in the rate and duration in time of wing development (HARNLY and HARNLY 1935). It must be remembered that mutations at this locus show changes in the form and amount of parts of the wing present, not changes in size only as in some of the other wing loci. The pattern theory advanced involved first growth longitudinally in the region of the II, III, and IV veins with no marked development in width beyond them, followed by growth mainly in the regions of the I and V veins, resulting in a pronounced increase in the width and area of the wing, the last part of the wing developed being the distal margin in the region of the III and IV veins. The same phenotype series has been found for the vestigial genotype in time of development.

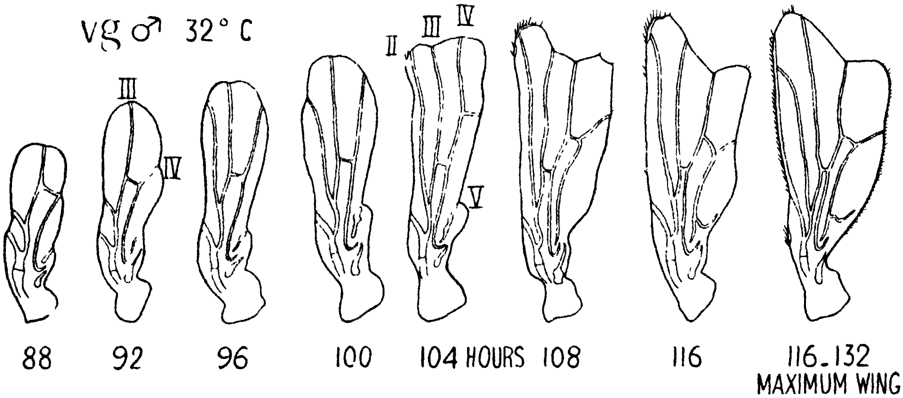

FIGURE 7

This pattern series in ontogeny is shown in figure 7. These wings were the mean types for males spending the number of hours indicated at 32° and completing their development at 25°. The wing at 88 hours was simply an enlarged vestigial wing with a length of 1.01 mm. It was equivalent to the wings of the homozygous vestigial males reared at 30° (HARNLY 1930a). Growth from then on was mainly longitudinal in the region of the II, III, and IV veins. Twelve hours later (100 hours) the wing was passing through a stage resembling the vestigial-strap mutant, many of the wings being indistinguishable from the published figures of strap wing. Four hours later (104 hours) the wings were similar to the allele antlered wing figured by MOHR (1932). Beyond this interval the bulk of the growth involved the regions of the I and especially the V veins with only minor increases in length. The maximum wing type was obviously a slight exaggeration of the vestigial allele notched-wing. The vestigial genotype during its development at 32° had passed in phenotype successively through the alleles vestigial, strap, antlered, and notched. The sequence of wing patterns for the females at 32° was the same, but had different time values since the rate was much slower in the females (see figures 1 and 2, 4 and 5). There was a definite pattern of wing development in time at 32°.

The same series can be demonstrated at 31°. The duration for the males at 31° and 32° was 48 hours but the rate at 31° was much slower. Consequently the mean wing attained at the end of the critical period was between the two wings shown for males at 32° of 100 and 104 hours of development. The period for the females at 31° was much shorter than that at 32° and the wings only reached a value between those of 88 and 92 hours for the males at 32° (figure 7). Changes in duration and rate with temperature changes had not affected the fundamental pattern of wing development; they simply determined the extent of the expression of that pattern in space. These changes in wing form with changes in duration or rate, together with the sequence of wing forms in time shown in figure 7, are in complete agreement with our hypotheses stated above and advanced recently (1935).

DISCUSSION

The facts established by the data presented here are: (1) beginning 1° below the critical temperature and extending through 29°, 30°, and 31° there were no significant changes in the duration of the embryonic, egg-larval or egg-larval-pupal periods; (2) their durations were practically identical for both sexes and all three temperatures; (3) the beginning of the critical period affecting wing formation at 30°, 31°, and 32° for the males and the females was the same (approximately 64 hours of total development); (4) the termination of this critical period varied with the sex and the temperature, its duration for the males being 20 hours at 30° and 48 hours at 31° and 32°, and for the females 8 hours at 30°, 20 hours at 31° and some undetermined time greater than 70 hours at 32°; (5) the rate of the developmental reactions involving the wings changed with temperature, that is, the duration for the males at 31° and 32° was 48 hours but the maximum rate at 32° was higher than that at 31° resulting in a much larger wing at 32°. With a constant developmental period for the whole organism, and a constant point in that period for the inception of the reactions involving the development of the wings, there were changes in both the duration and the rate of these reactions with changes in temperature. Obviously we are dealing here with an example of a differential effect of temperature on various, as yet undetermined, developmental processes. (6) From the wing types produced by homozygous vestigial, homozygous dimorphos vestigial, homozygous pennant, heterozygous pennant/vestigial, and dimorphos/dimorphos pennant/vestigial through the range of 16° to 32° and from the wings produced in the temperature transfers just discussed it has become evident that (a) there is a definite pattern of wing development in time, (b) the degree of expression is dependent on the duration and rate of processes occurring in the larval period of the vestigial genotype, and (c) apparently mutations at the vestigial locus affect the duration and rate of these developmental processes. Any attempted general interpretation of these facts must fit both the data presented here and the results of STANLEY (1931, 1935).

STANLEY (1931) reported on the temperature-effective periods at 17° and 27° C. for vestigial wing and recently (1935) on those at 30° and 31° for vestigial wing and 17°, 27°, and 30° for the wild type normal allele of vestigial wing together with the heterozygotes at 17° and 27° C. With two temperatures common to his work and the data presented here certain comparisons can be made and conclusions may be drawn. Both his work (1931) and mine (1930a) show the same critical temperatures for the vestigial wings of the males and for the wings of the females. But there are significant differences for wing length at the higher temperatures resulting in somewhat different curves; differences on the whole constant and consistent in all our experiments.

His wing length for total development of males at 29° was 0.98 mm and mine 0.74 mm; at 30° we obtained 1.52 mm and 1.00 mm respectively; and at 31° we differed by 1.64 mm and 1.70 mm. The same differences were again consistently present in the maximum mean wing length for the males in his transfer experiments from 30° and 31° to a lower temperature and in the data presented here for the transferred males at those temperatures. Similar consistent differences throughout were evident for the females. My own stock was inbred and selected, STANLEY'S stock was inbred but unselected. It is obvious, I believe, that we were dealing with different sets of sex-linked and autosomal genes modifying the action of the vestigial gene. The validity of such an assumption is indicated by STANLEY'S selection and tests at the end of his experiments (1935). He found that some of his heterozygous Wild/vestigial flies had "beaded" wings. From his original inbred and unselected vestigial S V A line he selected two new lines, S V B comparatively free of the modifying genes, and S V C practically homozygous for them. These three vestigial stocks were then tested at 29.5° and found to be significantly different in wing length. He says "Since 29.5° is very close to the 'critical temperature' for wing length, two assumptions are possible: (1) the critical temperature is shifted upward by the presence of the factors, or, (2) the factors have a direct inhibitory effect upon wing length. It is impossible, with the data available, to say which of these two assumptions is nearer the truth." In his case either may be correct. We have shown recently (HARNLY and HARNLY 1935) that the critical temperature of vestigial may be lowered as much as 5° by the presence of the sex-linked modifier dimorphos. From the consistency of the values obtained throughout his work and throughout mine and the evidence advanced for the presence of modifying genes we may assume that those points in which our results differ were due to the presence of different sets of modifying genes.

In view of those differences it is significant that we are in practical agreement on the moment in development at which temperature becomes operative. We have both found the first indications of this action in the vestigial flies (males and females) around 64–68 hours of development at 30° and 31° and I have found the same point at 32°. STANLEY reports 68 hours as the critical point at 30° for homozygous Long-winged flies. The similar time of inception of the critical period in development for the wings at these temperatures in the homozygous normal allele of vestigial and in homozygous vestigial with either of two sets of modifying factors would all indicate that its initiation was probably not due so much to the vestigial gene or its normal allele as to the reaching of some general stage or process in larval development. From CHEN'S work (1929) this stage morphologically cannot be either the first appearance of the dorsal mesothoracic buds, which comes much earlier in development, nor the first appearance of the wing buds which comes much later in ontogeny. The work of ALPATOV (1929, 1930a) on the development of wild and vestigial larvae indicates that the most probable morphological point of attack is the molt between the second and third instars. Tentatively then I am assuming that the critical period in wing development at these high temperatures is initiated in time by the molt from the second to the third instar. This assumption has one major advantage over the others that might be advanced, the facility with which it may be proved or disproved. By the use of a new technique it is hoped to substantiate or invalidate this assumption in the determination of the temperature-effective period for pennant, a new regressive mutation from vestigial to a recessive wild or normal type wing.

Our close agreement on the beginning of the temperature-effective period together with both the marked differences in our end points in time at 30° and 31°, and the pronounced changes I have found in the time of termination at 30°, 31°, and 32°, indicate that the beginning and end of this period were determined by different and independent processes. Furthermore, as I have shown above, the processes ending this critical period were not associated with the effects of temperature on the general rate of development. Without any change in either the length of the egg-larval period or the time at which the temperature-effective period began at 30° and 31°, there was an increase of 140 percent in the duration of the critical period with this rise of 1° for the males of our vestigial stock. Similar results were obtained for the females. This rise of 1° in some way enabled the vestigial gene to continue its activities much longer in association with the rest of the gene complex controlling those processes leading to wing development. This interpretation agrees with the assumption that the vestigial mutation produced a gene operating within the refractory temperature limits for a much shorter interval of time and a different rate from its normal allele, consequently producing during ontogeny less of the precursor of the ultimately formed wing bud. The differences in the termination points and the duration of the egg-larval periods as related to the duration of the temperature-effective periods determined at 30° and 31° by STANLEY and myself were probably due to the different sets of modifiers present in our vestigial stocks. That even a single modifier may have a profound effect on the action of the vestigial gene was shown by the fact that the mutant sex-linked modifier dimorphos enabled the vestigial gene to produce wild-type wings at these high temperatures (HARNLY and HARNLY 1935). We may conclude that the initiation of the temperature-effective period at these high temperatures was dependent on the attainment of some developmental stage (probably the molt from the second to the third instar) and that its termination was independently determined by the response of the wing gene complex to specific temperatures, the consequent durations in my stock being disproportional to the duration of the larval periods in which they occurred. The rates of the processes concerned with wing formation were likewise dependent on or related to the temperature experienced during the critical period of development. Any attempt to explain further the action of the vestigial gene and its relation to temperature would be hazardous at this time. The responses of its allele pennant and the heterozygote pennant/vestigial in total development through the temperature range of 16° to 32° preclude any explanation in terms of the data available at present. These experiments with pennant and pennant/vestigial have been completed and will be reported shortly.

GOLDSCHMIDT in the course of his extensive studies on *Lymantria dispar* presented in 1920 a general theory of the action of the genes in development, a theory which he has evolved more completely since then (GOLDSCHMIDT 1934, complete bibliography). This theory is based on his work on intersexuality and pigmentation patterns in the larvae and assumes that the genes affect the rates and durations of processes in ontogeny. The data presented here on vestigial are in agreement with his hypothesis.

SUMMARY

1. The temperature-effective periods for the length and area of the vestigial wings of an inbred selected stock of *D. melanogaster* are reported for 30°, 31°, and 32°.
2. The critical period began at approximately 64 hours for both sexes at these temperatures. It had a duration of 20 hours at 30° and 48 hours at 31° and 32° in the males and 8, 20, and some interval more than 70 hours for the females. Rises of 1° at these temperatures did not affect the inception of the period but markedly affected its termination and the rate; that is, in the males at 31° and 32° with no change in duration there was a marked increase in the rate.
3. The differences between the sexes in duration and rate with increases of 1° afford a formal explanation of the sexual dimorphism of the wings.
4. A complete reversal of the relative sizes of the wings of the males and the females was found at 33° for all developmental points examined.
5. There was never any close approach to the wild type wing at these high temperatures. The growth pattern of wing form in time is shown and related to the alleles at the vestigial locus and the theory of wing pattern and mutation stated in an earlier paper.
6. The duration of the embryonic, egg-larval, and egg-larval-pupal periods were determined at 25°, 29°, 30°, 31°, and 32°. Pronounced differences in the length of these periods were found between 25° and 29°. No significant changes occurred in their duration at 30° and 31°. The effects of temperature above 29° on the duration of the major periods of development offer no explanation of the critical temperatures, the sexual dimorphism, or the increase in wing size. The results must be due to a differential effect of temperature on various processes during ontogeny.
7. The inception of the critical period in development is interpreted as occuring at the molt from the second to the third instar at these high temperatures; its termination is apparently independently determined by the gene complex (including sex) and the temperature experienced, the consequent durations being disproportional to the durations of the larval periods. The rate is likewise dependent on the gene complex and the temperature.

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THE INHERITANCE OF SUSCEPTIBILITY TO YELLOW FEVER ENCEPHALITIS IN MICE

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INTRODUCTION

THE importance of host resistance as a factor in determining the incidence of infectious disease has been emphasized in recent studies. The methods developed in the study of genetics have provided a practical means of investigating the problems of host resistance and from the accumulated data there can be no doubt of the existence of heritable factors which affect the response of the individual to infection. A bibliography of the more important contributions in this field is given in a review of the subject by HILL (1934). The experiments to be reported here give evidence of the presence of such factors influencing the incidence of a virus disease, yellow fever.

The susceptibility of mice to the virus of yellow fever was demonstrated by THEILER (1930). SAWYER and LLOYD (1931) tested the suitability of mice as test animals in the study of yellow fever immunity and found that different strains of mice varied in susceptibility to this virus, ranging from 100 percent to 50 percent mortality with standard dosage of virus. This information was not only of practical value in the work on yellow fever immunity, but also indicated the operation of genetic factors in yellow fever resistance. On this basis the following investigation was undertaken.

METHOD OF TESTING

Since yellow fever infection occurs in mice only as an encephalitis, test inoculations were made by intracerebral inoculation while the mice were under ether anaesthesia. Susceptible mice when so inoculated with a virus "fixed for mice" show a typical paralysis which usually occurs on the fourth or fifth day after inoculation and terminates in death within one day from onset. Mice showing no paralysis in ten days are classed as survivors and are discarded.

The virus selected had been "fixed" by passing in mice from brain-to-brain for over 100 passages and had reached its maximum virulence for this animal. To obtain approximately equal dosages, a virus was used which had been preserved by drying while in the frozen state, in which form SAWYER, LLOYD, and KITCHEN (1929) have shown its virulence to be retained over a period of years. The dried virus was diluted with a sufficient quantity of suitable diluent to yield a final concentration in which approximately 30 minimum lethal doses were contained in 0.03 cc., which amount is inoculated intracerebrally into the mice to be tested. However, for certain technical reasons concerned with the drying of virus, tubes opened at different times vary in virus content, and since the keeping quality of the virus is such that titrations cannot be completed before the virus activity is lost, the doses are not uniform. To overcome this difficulty the contents of several tubes are pooled for each test and to demonstrate viability of the virus a number of unselected Swiss mice are inoculated with each virus suspension used.

MATERIAL

The "Swiss" strain was chosen as a source of susceptible animals. The foundation stock consisted of 2 males and 7 females obtained in 1926 from Dr. A. de Coulon in Lausanne, Switzerland. The individuals used in these experiments were taken from lines, some of which were inbred brother by sister and others partly pen inbred. SAWYER and LLOYD (1931) had previously tested mice from a branch of this stock, observing 100 percent mortality in each of two lots consisting of 30 and 23 mice respectively.

TABLE 1

Mortality percentages of mice from the Swiss and Det strains. Preliminary tests

TESTED BY	GROUP	NUMBER TESTED	NUMBER DEAD	PERCENT DEAD	PERCENT DIFFERENCE	χ^2	P
Sawyer and Lloyd	Swiss (test 4)	30	30	100 0			
	Swiss (test 5)	23	23	100.0	18.5	10.83	<.01
	Det (test 3)	54	44	81.5	15.6	4.01	.05
Lynch and Hughes	Det	88	58	65.9	31.2	40.91	<.01
	Swiss	138	134	97.1			

The strain selected as a source of resistant stock had also been tested by SAWYER and LLOYD. Although in their tests the lowest mortality had been given by the "Rockefeller Institute Stock," certain practical considerations led to the choice of stock referred to by SAWYER and LLOYD as "Strain D" and designated by us as "Det." Five pairs were purchased and they and their descendants were inbred, usually brother by sister, but occasionally parent by offspring. Mice from the second to the sixth generations were used in these tests.

In the hands of SAWYER and LLOYD, the Det mice had a mortality of 81.5 percent (table 1), about 18.5 percent lower than the Swiss strain; however, the comparison may not be accurate since the two groups were inoculated with different virus preparations. A preliminary test of the Det mice in our colony resulted in a distinctly lower figure: of 88 mice inoculated, 65.9 percent succumbed. Among 138 Swiss mice tested with the identical virus preparation, there was 97.1 percent mortality. The difference is 31.2 percent and the χ^2 test for the significance of the difference gives P as $<.01$. This result confirmed our choice of material since the divergence of the strains is sufficiently great to warrant a cross between them. Therefore matings were made between representatives of the Det and Swiss strains and the hybrids thus produced were crossed back to the parental strains, sometimes to the original parents. The resulting population was divided into two lots and was inoculated with virus. Variations in the rate of reproduction together with exigencies of time and laboratory space prevented a regular scheme of distribution into two groups so parents and their offspring were not always inoculated together. During the course of the experiment some animals died of intercurrent disease before their reaction to yellow fever virus could be determined. Not all individuals of the P_1 and F_1 generations contributed offspring to the experiment.

RESULTS

The first test included samples from all the generations under observation (table 2). The original strains were represented by 39 Swiss and 18 Det mice. Upon inoculation all the Swiss mice succumbed, while the mortality rate of the Det mice was 77.8 percent. The difference between the strains, when measured by the χ^2 test is significant, $\chi^2=9.32$ and P $= <.01$. Thirty-seven individuals were available for examination in the F_1 generation obtained from the cross Swiss by Det. Of these 31, or 83.8 percent died—a mortality not much higher than that of the Det and significantly different from that of the Swiss strain ($\chi^2 = 6.86$; $P = <.01$). In the backcross generation somewhat larger numbers are dealt with, the two classes comprising 146 and 99 animals respectively. The descendants produced by crossing the hybrid to the more susceptible strain showed a heightened mortality (93.8 percent) while the mice resulting from the

TABLE 2

Test I. Mortality percentages of representatives of the parental strains, F_1 and backcross progeny

GROUP	NUMBER TESTED	NUMBER DEAD	PERCENT DEAD	PERCENT DIFFERENCE	χ^2	P
Swiss	39	39	100.0	22.2	9.32	<.01
Det	18	14	77.8			
F_1 (Swiss×Det)	37	31	83.8			
Backcross (F_1×Swiss)	146	137	93.8	23.1	24.08	<.01
Backcross (F_1×Det)	99	70	70.7			

backcross to the less susceptible stock showed a decreased mortality (70.7 percent). This difference is greater than would be expected on a basis of random sampling of the same population, having a χ^2 value of 24.08 and $P = <.01$.

The second test also included representatives of the five classes (table 3). Only 8 Swiss mice were available but again the mortality was 100 percent, while 61 mice from the Det strain showed only 50.8 percent mortality—the lowest rate yet observed. The F_1 had a mortality of 65.4 percent. Compared with the Swiss strain the difference in mortality is probably significant ($\chi^2 = 3.76$; $P = .05$). The backcross progeny produced by mating the F_1 hybrids to the more susceptible strain showed a mortality of 70.2 percent while in the backcross to the less susceptible strain the mortality was reduced to 50.0 percent. The difference between them is 20.2 percent, about the same as in the first experiment, and is also mathematically significant ($\chi^2 = 8.32$ and $P = <.01$).

TABLE 3

Test II Mortality percentages of representatives of the parental strains, F_1 and backcross progeny

GROUP	NUMBER TESTED	NUMBER DEAD	PERCENT DEAD	PERCENT DIFFERENCE	χ^2	P
Swiss	8	8	100.0	49.2	6.96	<.01
Det	61	31	50.8			
F_1 (Swiss×Det)	26	17	65.4			
Backcross (F_1×Swiss)	114	80	70.2	20.2	8.32	<.01
Backcross (F_1×Det)	84	42	50.0			

In comparing the two tests certain similarities are apparent. In both cases there is a significant difference between the susceptible and partially resistant stocks, the hybrids are more like the resistant strain and the backcross groups have a heightened or lowered mortality depending on the degree of susceptibility of the parent stock to which the backcross was made. On the other hand, the dissimilarities are so marked that it becomes doubtful whether the figures of the two tests should be combined. Each class in the second test has a lower mortality than its corresponding set in the first test, with the exception of the Swiss in which only 8 individuals were used in the second test. The samples of the parental Det strain gave significantly different mortalities in the two tests and the backcross progeny from the susceptible strain showed no greater mortality in the second test than did the backcross progeny from the resistant strain in the first test. Since in all probability the quantity of virus in the inoculum varied between tests, the lower mortality rate in the second test may reasonably be referred to this difference in virus content. However, a further genetic analysis of the data is possible. Some of the hybrid parents were backcrossed in the two directions. We find that, in the first experiment 4 F_1 parents, and in the second test 6 F_1 parents were mated with individuals from each stock. Their offspring are listed in tables 4 and 5. These tables show in detail the degree of regularity with which these individuals produced offspring with unlike mortality ratios among siblings resulting from contrasted matings. The totals again indicate hereditary differences.

TABLE 4

Test I. Mortality rates of the progeny of the same F_1 individual backcrossed to both parental strains

IDENTIFICATION NUMBER OF F_1 PARENT	BACKCROSS PROGENY				SIGNIFICANCE OF DIFFERENCE
	FROM F_1×DET		FROM F_1×SWISS		
	NUMBER DEAD	NUMBER ALIVE	NUMBER DEAD	NUMBER ALIVE	
♂ 69.0	8	6	14	0	
♂ 70.0	5	2	22	1	
♀ 54.1	5	1	6	0	
♀ 54.3	4	0	5	0	
Total	22	9	47	1	$\chi^2 = 12.37$ P = < .01
Percent dead	71.0		97.9		

TABLE 5

Test II. Mortality rates of the progeny of the same F_1 individual backcrossed to both parental strains

IDENTIFICATION NUMBER OF F_1 PARENT	BACKCROSS PROGENY				SIGNIFICANCE OF DIFFERENCE
	FROM F_1×DET		FROM F_1×SWISS		
	NUMBER DEAD	NUMBER ALIVE	NUMBER DEAD	NUMBER ALIVE	
♀ 68.20	0	5	10	0	
♀ 55.40	1	2	3	0	
♀ 55.25	1	4	7	5	
♀ 61.46	0	11	10	2	
♂ 100.00	6	12	9	8	
♂ 101.00	1	0	6	3	
Total	9	34	45	18	$\chi^2 = 26.08$ P = < 01
Percent dead	20.9		71.4		

A more exact comparison may be made between the offspring of the various types of mating when the susceptibility of both parents is known. The animals for which this information is available are grouped in table 6. In the first test all of the immediate progeny in any generation resulting from crosses between two susceptible parents may be compared with the progeny resulting from matings in which only one parent was susceptible. There were only 9 mice available in the second class but their mortality was 22.2 percent as contrasted with a mortality of 91.4 percent among those with two susceptible parents. In the second test the figures for the corresponding classes are 27.6 percent and 69.6 percent, again a significant difference. In addition there was a third class comprised of 15 individuals with two resistant parents. In this group there was but one death, a mortality rate of 6.7 percent; however, this figure is not significantly lower than that given by the offspring from unlike parents.

TABLE 6

Mortality percentages among mice classified as to whether both parents were susceptible, only one susceptible, or both resistant

TEST	PARENTAGE	NUMBER DEAD	NUMBER ALIVE	PERCENT DEAD	PERCENT DIFFERENCE	χ^2	P
I	S×S	74	7	91.4	69.1	29.47	<.01
	S×R	2	7	22.2			
II	S×S	16	7	69.6	42.0	9.10	<.01
	S×R	8	21	27.6	20.9	2.66	.10
	R×R	1	14	6.7			

S = Susceptible.
R = Resistant

By this classification also the values obtained in the two tests are not similar. In the first test the mortality in the population derived from susceptible parents was 91.4 percent while in the second test it was only 69.6 percent. Since it has not been shown that the parental matings were between homozygotes, the genetic expectation for these values cannot be computed. However, as a whole the data seem sufficient to justify the conclusion that the type of parentage and the susceptibility of the offspring are correlated.

On the assumption that the two tests may properly be treated as self-contained units, an inquiry into the homogeneity of the groups of which they were composed was undertaken. The Lexian ratio was used to test the dispersion of the probabilities of death among the litters in each group, although the numbers dealt with in some cases were small. In the preliminary study of the Swiss, the sibling relationships were known for only 96 mice but this number which contained the only four resistant animals of the group proved homogeneous (table 7). While the Det stock appeared uniform in the first test, all of the mice used as progenitors were not tested and the larger population used in the second test was not homogeneous. The F_1 hybrid generation gave different results in the two tests. The backcross progenies lacked uniformity but homogeneity was shown in the groups classified on a basis of parentage.

TABLE 7

Tests for the type of dispersion of the probabilities of death within various groups

TEST	GROUP	NUMBER TESTED	NUMBER OF FAMILIES	L	χ^2	P
Preliminary	Det	88	27	1.22	40.27	.04
	Swiss	96	35	1.10	42.59	.18
I	Swiss	39	—	—	—	—
	Det	18	6	1.11	7.39	.19
	F_1 (Swiss$\times$Det)	37	7	1.41	13.85	.04
	Backcross ($F_1\times$Swiss)	146	20	1.71	59.06	<.01
	Backcross ($F_1\times$Det)	99	20	1.52	45.94	<.01
II	Swiss	8	—	—	—	—
	Det	61	25	1.35	45.60	<.01
	F_1 (Swiss$\times$Det)	26	7	1.12	8.73	.19
	Backcross ($F_1\times$Swiss)	114	22	1.35	40.27	.01
	Backcross ($F_1\times$Det)	84	22	1.63	58.58	<.01
I	S$\times$S	81	13	0.77	7.66	.81
	S$\times$R	9	2	0.40	0.32	.59
II	S$\times$S	23	5	1.16	6.84	.15
	S$\times$R	29	8	1.01	8.14	.32
	R$\times$R	15	3	1.20	4.29	.12

In table 8 the various groups are arranged according to sex. There is no indication that sex is a differentiating factor. The apparently greater susceptibility of females shown in the last backcross group where a test for independence gives χ^2 as 8.16 and P as <.01, is probably due to the fact that in several litters where resistance was high there were no sisters brought to test.

TABLE 8

Comparison of mortality of the sexes within various groups

TEST	GROUP	SEX	NUMBER DEAD	NUMBER ALIVE
I	F_1 (Swiss$\times$Det)	♂♂	15	4
		♀♀	17	1
	Backcross ($F_1\times$Swiss)	♂♂	55	3
		♀♀	82	6
	Backcross ($F_1\times$Det)	♂♂	30	16
		♀♀	40	13
II	F_1 (Swiss$\times$Det)	♂♂	10	6
		♀♀	7	3
	Backcross ($F_1\times$Swiss)	♂♂	44	19
		♀♀	36	15
	Backcross ($F_1\times$Det)	♂♂	12	25
		♀♀	30	17

DISCUSSION

In attempting to demonstrate genetic factors, it becomes necessary to control as far as possible any environmental conditions which may affect the results of the tests. The immediate surroundings in the laboratory were kept as constant as was possible, but many variables exist which are beyond control. In this experiment the occurrence of acquired immunity may safely be ruled out, since the mice were sent to a laboratory remote from the breeding room for tests. No survivors were returned. There can be no question as to the freedom of the tested animals from previous contact with yellow fever virus.

No selection of strains was undertaken during this experiment. Rather, the investigation was planned to take advantage of strains from diverse sources in which some differentiation already had occurred. Although lack of known material for the various crosses precludes a final analysis, the existence of segregating factors for susceptibility and resistance is evident. This is clearly shown by the differential ratios given first by backcrosses and second by progeny from different sorts of matings. The latter criterion for inheritance is not of universal application. When in a genetically pure strain, the character in question, because of somatic fluctuation, does not manifest itself with absolute regularity, progeny from a mixed parentage do not differ significantly from offspring descended from like parents. If progeny groups are dissimilar, whether the parents do or do not present phenotypic differences, they must be different genetically. Consequently progeny are the most reliable basis for determining the constitution of parents. When differing groups of progeny are descended from unlike types of matings, as in the present case the genetic explanation is self-evident.

As to the mode of inheritance, since matings between like parents, either both susceptible or both non-susceptible, produced two types among their offspring either fluctuating variations or the joint occurrence of a number of genes are indicated. Neither of these alternatives can be ruled out by our data.

SUMMARY

1. Mice from two sources gave different mortality rates when inoculated with the virus of yellow fever. This difference was maintained in three separate tests.

2. When the strains were crossed, the hybrids showed a mortality less than that of the susceptible strain. In one test the difference was clearly significant; in another it was probably significant. By crossing the hybrid back to the susceptible strain the mortality rate was increased; by crossing back to a more resistant strain the rate was lowered. This relationship was demonstrated in two tests.

3. When the mice were classified according to parentage, offspring from two susceptible parents were more susceptible than were offspring with one or two resistant parents.

4. Susceptibility did not appear to be modified by sex.

5. It is concluded that hereditary factors for resistance to yellow fever encephalitis are present in mice.

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STUDIES ON HYBRID STERILITY. II. LOCALIZATION OF STERILITY FACTORS IN DROSOPHILA PSEUDOOBSCURA HYBRIDS

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THE PROBLEM

THE causation of hybrid sterility has long been one of the unsolved problems of biology. To date, probably the greatest advance in this field has been made by FEDERLEY who discovered a failure of the meiotic chromosome pairing in the sterile hybrids between moths of the genus Pygaera. This finding has since been amply corroborated by other investigators in sterile hybrids both in animals and in plants. Naturally enough it became tempting to suppose that the failure of the meiotic pairing is the cause of hybrid sterility. The restitution of the normal meiotic pairing as well as of fertility following the reduplication of the chromosome complement in allotetraploid hybrids seems to be further evidence in favor of this view. And yet, this view proves to be inadequate as a general explanation of hybrid sterility. Two difficulties deserve particular attention. First, some hybrids are sterile despite the fact that the meiotic pairing in their gametogenesis is apparently normal (MICHAELIS 1933 in Epilobium, DOBZHANSKY 1934 in some crosses in *Drosophila pseudoobscura*); while in other sterile hybrids the gametogenesis does not reach the meiotic stages (KERKIS 1933, in *Drosophila melanogaster* × *D. simulans*). Second, the failure of the meiotic pairing in sterile hybrids is usually attributed to an "incompatibility" of the chromosomes of species or races entering the cross. This, clearly, is a restatement of facts and not a causal explanation. It remains possible that suppression of meiotic pairing may be caused by different mechanisms in different cases, and that sometimes there is no cause and effect relation between the failure of pairing and the sterility.

In my previous publications (DOBZHANSKY 1933, 1934) a hypothesis was suggested according to which there exist at least two different types of hybrid sterility—the chromosomal type and the genic type. The chromosomal type is caused by differences in the gross structure (gene alignment) of the chromosomes of the parental forms, preventing, through competition in pairing, the normal conjugation of the chromosomes at meiosis, and causing their irregular disjunction. The genic type of sterility is due to interactions between complementary genetic factors contributed by both parents. If the genetic constitution of one of the parental forms is *SStt*, and of the other *ssTT*, the hybrid is *SsTt*. The assumption is made that the presence of the factor (or the group of factors) *S* alone, or of the factor *T* alone, permits unlimited fertility, but that the factors *S* and *T* interact in such a manner as to make sterile an organism carrying them simultaneously.

Although some indirect evidence for the existence of these two sterility producing mechanisms is available (cf. DOBZHANSKY 1934), the above hypothesis cannot yet be considered proved. To date, in no case has a sufficient amount of gross structural differences between chromosomes of related species been demonstrated, nor have the theoretically postulated sterility genes been isolated and localized. The experiments to be reported in the present article are aimed at securing some information on this subject. The sterility of the male hybrids between race A and race B of *Drosophila pseudoobscura* is apparently of the genic type, (DOBZHANSKY and BOCHE 1933, DOBZHANSKY 1933, 1934). The results of LANCEFIELD (1929) and of KOLLER (1932) suggest that some of the differences producing sterility between these races are located in the X-chromosome, while autosomes seem also to be involved (DOBZHANSKY 1933a). Further attempts to localize the sterility genes in the chromosomes of *Drosophila pseudoobscura* are described below.

The writer wishes to acknowledge his obligations to Professors C. V. BEERS (Los Angeles), F. A. E. CREW and P. C. KOLLER (Edinburgh) for permission to use some of the mutants discovered by them in *D. pseudoobscura* the descriptions of which are not yet published. Thanks are due also to Doctor A. H. STURTEVANT for many valuable suggestions and criticisms.

METHOD

Factors whose interaction is responsible for the sterility of a hybrid are a part of the germ plasm, and hence must have a physical carrier in the gametes. The task is to find out in which of the constituents of the gametes these factors are localized. Races A and B of *D. pseudoobscura* produce in F_1 completely sterile male hybrids, but fertile females. The latter may be back crossed to males of either parental race. In the offspring of the backcrosses various combinations of the chromosomes and of the cytoplasms of the ancestral forms should occur in separate individuals. Some of the individuals may be expected to be sterile, and others fertile. Provided the chromosomes are marked by appropriate mutant genes, the genetic structure of a given individual may be recognized by its phenotype. Hence, it will be possible to determine which combinations of the ancestral elements are necessary to induce sterility, and which permit the individual to be fertile.

In practice, two experimental procedures are possible. First, by repeatedly backcrossing hybrid females to males of the same race, one may secure individuals carrying one of the elements (e. g., a chromosome or a part thereof, or the cytoplasm) of one race, and all other elements from the other race. Second, among the male offspring of the first backcross generation individuals should be found representing many of the possible combinations of the above elements Both experimental procedures should, probably, be made use of before a final solution of the problem here under consideration may be consummated. The data reported in the present article are based on the results of the application of the second procedure. The conclusions arrived at are, however, in accord with those suggested by the outcome of the few experiments of Doctor A. H. STURTEVANT and the writer involving the application of the first procedure.

Males appearing in the progeny of the backcrosses have testes ranging in size from normal (i.e., that found in males of either parental race) to very small. Males having small testes are invariably sterile, those with testes of normal or nearly normal size are mostly fertile. It has been shown cytologically (DOBZHANSKY 1933, 1934) that the disturbances of the spermatogenesis leading to sterility are greatest in the very small testes, and least in large ones. Hence, size of the testis is a fair measure of the degree of departure from the normal structure and functioning of the testis. This fact is very important for our purposes, since it permits a sort of quantitative expression of the results of investigation of the hybrid males. The smaller the testes in males of a given genetic constitution, the more (or the stronger) sterility factors they carry.

TECHNIQUE

Testis size in *D. pseudoobscura*, particularly in the hybrid males, is exceedingly sensitive to environmental factors. Although our conclusions are to be based on a comparison of testis size in different classes of males developed in the same culture bottles, and hence under identical environment, care was taken to insure homogeneous culture conditions in all experiments. Three to four F_1 hybrid females were placed in the same bottle with five to six males. Parents were kept in vials with food for four days, transferred (without etherization) to standard culture bottles, placed in incubator at 24.5° C., and allowed to oviposit for about four days, transferred to fresh culture bottles, and again left there for a similar length of time.

When the progeny of the backcross started to emerge, flies were classified according to the marking genes they carried, and males were dissected in physiological salt solution. Testes were isolated, and their length measured with the aid of an eyepiece micrometer (1 unit $=17.4\mu$). The technique of the measurements has been described by DOBZHANSKY and BOCHE (1933). The testis of *D. pseudoobscura* is ellipsoidal in shape; its greater diameter shows a correlation with its shorter diameter, so that either of these measurements gives a fair idea of the size of the whole organ. However, in the adult males the shape of the testis changes somewhat with age, becoming relatively longer and more slender. To avoid this source of error, cultures were examined every second day, and males that had hatched in the meanwhile were dissected and measured immediately. Only males that emerged during the first 6 to 8 days of hatching in a given culture bottle were used.

The statistical data obtained are far too voluminous to be published in detail under the present conditions; they are, of course, preserved, and open to all interested. The statistical constants were calculated only for classes in which ten or more individuals were measured.

PRELIMINARY EXPERIMENTS

Theoretically, sterility factors might be located in any of the constituents of the gametes. A few simple experiments of exploratory nature were undertaken to narrow the range of possibilities by excluding some of them as inadequate to explain the situation.

If F_1 hybrid females from the cross A ♀ × B ♂ are used for making backcrosses, the resulting progeny has race A cytoplasm. By crossing such females to race B males, some of the males appearing in the next generation should have all race B chromosomes in race A cytoplasm. If the sterility depends upon an interaction of the chromosomes of one race with the cytoplasm of the other, such males should be sterile. Actually in a number of tests they were found to be fertile. Similarly, males having race A chromosomes in race B cytoplasm proved fertile. This shows that at least the most important sterility factors are not located in the cytoplasm.

To exclude the influence of the Y chromosomes is more difficult. The males devoid of the Y chromosome (XO males) in pure races are sterile, but the structure of their testes shows no similarity whatever with that of the sterile hybrid males (DOBZANSKY 1933). This is in agreement with the results of SHEN (1932) who found that the sterility of XO males in *D. melanogaster* is caused by disturbances in the vesiculae seminales rather than in the testes. Some hybrid males in the backcrosses of hybrid females to race A males are likewise XO. These are, of course, always sterile, but the size, as well as the internal structure of their testes, is variable, just as in their XY sibs. This variability can be due only to the chromosomes other than the Y carried by a given male (DOBZHANSKY 1933). This makes the assumption of factors responsible for hybrid sterility in the Y chromosome unnecessary.

LANCEFIELD (1929) and KOLLER (1932) found that males carrying an X chromosome of one race and a majority of the autosomes of the other race have small testes and are sterile. Furthermore, by studying crossovers in the X chromosome they found that both ends (or, rather, both limbs) of the X chromosome are concerned with the sterility, while the middle part is apparently not involved. Thus the location of some of the sterility factors was established.

STURTEVANT and the writer attempted to "transfer" mutant genes known in one race into the other race. For this purpose females carrying a given gene, for instance of race A, are repeatedly backcrossed to males of race B, until individuals are obtained possessing all chromosomes of race B except for a more or less short section containing the genes in question. These experiments showed that some of the mutant genes can be thus transferred from race to race, and males carrying these "foreign" genes become fertile after from one to several generations of backcrosses. To this class belong the genes Pointed and short (in the X chromosome of race A), Smoky (second chromosome of race B), Curly (fourth chromosome of B). It follows that sections of chromosomes including these genes contain either no factors concerned with sterility, or else these factors are not by themselves sufficient to produce sterility (although they may, perhaps, do so in combinations with other genes). On the other hand, the attempts to transfer the genes Bare (A) and cinnabar (B) (second chromosome) from one race to another have so far given negative results despite the numerous backcrosses. Similarly, Pointed proved to be closely linked with a sterility-producing section of the X chromosome, but it can be separated from this section by crossing over. Thus, sterility-producing genes were found in both the X chromosome and in an autosome (the second chromosome). The data presented below corroborate this conclusion.

Backcross to race A

Race A females carrying the sex-linked recessives beaded (*bd*), yellow (*y*), and short (*s*), the second chromosome dominant Bare (*Ba*), and the third chromosome recessive purple (*pr*) were crossed to race B males carrying the third chromosome recessive orange (*or*) and the fourth chromosome dominant Curly (*Cy*). F_1 hybrid females heterozygous for these genes were selected and backcrossed to race A males homozygous for *or* and *pr*. The males coming from this backcross were classified for all of the above genes.

According to the setting of the experiment, every one of the chromosomes of the F_1 females (with the exception of the small fifth chromosome in which no genes are available) is marked by one or more genes, which should make the different classes of the backcross males distinguishable from each other phenotypically. Unfortunately, the control of all the chromosomes which is thus attained is far from complete, due to crossing over which takes place in the hybrid females. The X chromosome is marked by three genes, the third by two, and the second and fourth by only one each. The scarcity of marking genes is mitigated by the fact that the X, second, and third chromosomes of race A differ from the corresponding B chromosomes by inverted sections (TAN 1935 a, b) which suppress a part of the normal crossing over. Nevertheless, some crossovers undoubtedly escape detection.

TABLE 1

Length of the testis (in μ) in the offspring of the cross:
(bd y s Ba pr Race A ♀ × *or Cy Race B* ♂) F_1 ♀ × *or pr Race A* ♂.

PHENOTYPE	M±m	n	PHENOTYPE	M±m	n
1 *bd y s Ba pr*	668.2	9	33 *bd y Ba pr*	348.0	1
2 *bd y s Ba pr Cy*	563.8	8	34 *bd y Ba py Cy*	342.8	3
3 *bd y s Ba or*	585.9±14.9	23	35 *bd y Ba or*	478.5	6
4 *bd y s pr*	632.8±11.0	30	36 *bd y pr*	504.6	4
5 *bd y s Ba or Cy*	509.6±17.6	19	37 *bd y Ba or Cy*	407.2	9
6 *bd y s pr Cy*	602.7± 3.4	28	38 *bd y pr Cy*	435.0	3
7 *bd y s or*	551.2± 6.1	119	39 *bd y or*	462.8	8
8 *bd y s or Cy*	526.5± 8.9	66	40 *bd y or Cy*	418.1±24.4	17
9 *or Cy*	123.9± 2.8	336	41 *s or Cy*	290.2±18.1	22
10 *or*	113.1± 3.2	353	42 *s or*	310.9±18.6	27
11 *pr Cy*	94.0± 3.6	152	43 *s pr Cy*	231.4	4
12 *Ba or Cy*	66.6± 4.7	69	44 *s Ba or Cy*	191.4	1
13 *pr*	68.4± 3.7	159	45 *s pr*	187.9	5
14 *Ba or*	60.2± 4.6	71	46 *s Ba or*	34.8	1
15 *Ba pr Cy*	51.7± 5.9	38	47 *s Ba pr Cy*		
16 *Ba pr*	23.8± 7.3	23	48 *s Ba pr*		
17 *bd y s Ba*	640.3	5	49 *bd y Ba*	461.1	2
18 *bd y s Ba or pr*	664.7	4	50 *bd y Ba or pr*		
19 *bd y s Ba Cy*	553.3	5	51 *bd y Ba Cy*		
20 *bd y s Ba or pr Cy*			52 *bd y Ba or pr Cy*		
21 *bd y s*	597.2±10.4	22	53 *bd y*	574.2	1
22 *bd y s or pr*	617.7±18.4	10	54 *bd y or pr*	614.2	3
23 *bd y s Cy*	559.8±15.6	24	55 *bd y Cy*	461.1	2
24 *bd y s or pr Cy*	494.2	6	56 *bd y or pr Cy*	626.4	1
25 *Cy*	122.8± 4.8	82	57 *s Cy*	274.9	4
26 *or pr Cy*	94.1±10.3	35	58 *s or pr Cy*	487.2	1
27 wild-type	110.7± 5.0	86	59 *s*	261.0	6
28 *or pr*	69.8 ± 6.7	33	60 *s or pr*	104.4	1
29 *Ba Cy*	43.5± 7.3	19	61 *s Ba Cy*		
30 *Ba or pr Cy*	34.8	5	62 *s Ba or pr Cy*		
31 *Ba*	17.1± 4.6	21	63 *s Ba*	87.0	2
32 *Ba or pr*	47.0	3	64 *bd or*	574.2	1

In spite of the above difficulty, it is believed that the control of the chromosomes, incomplete as it is, is on the whole adequate for our purpose, namely testing for the presence or absence of the sterility genes in each of the chromosomes covered by the investigation. The basis of our judgement is the mean testis size in the males of a given class showing a given combination of the marking genes. Although some of the chromosomes are marked by a single gene only, all the males carrying this gene will carry at least a part of the chromosome of the race indicated by this gene, and the majority will carry probably the entire chromosome. In any event,

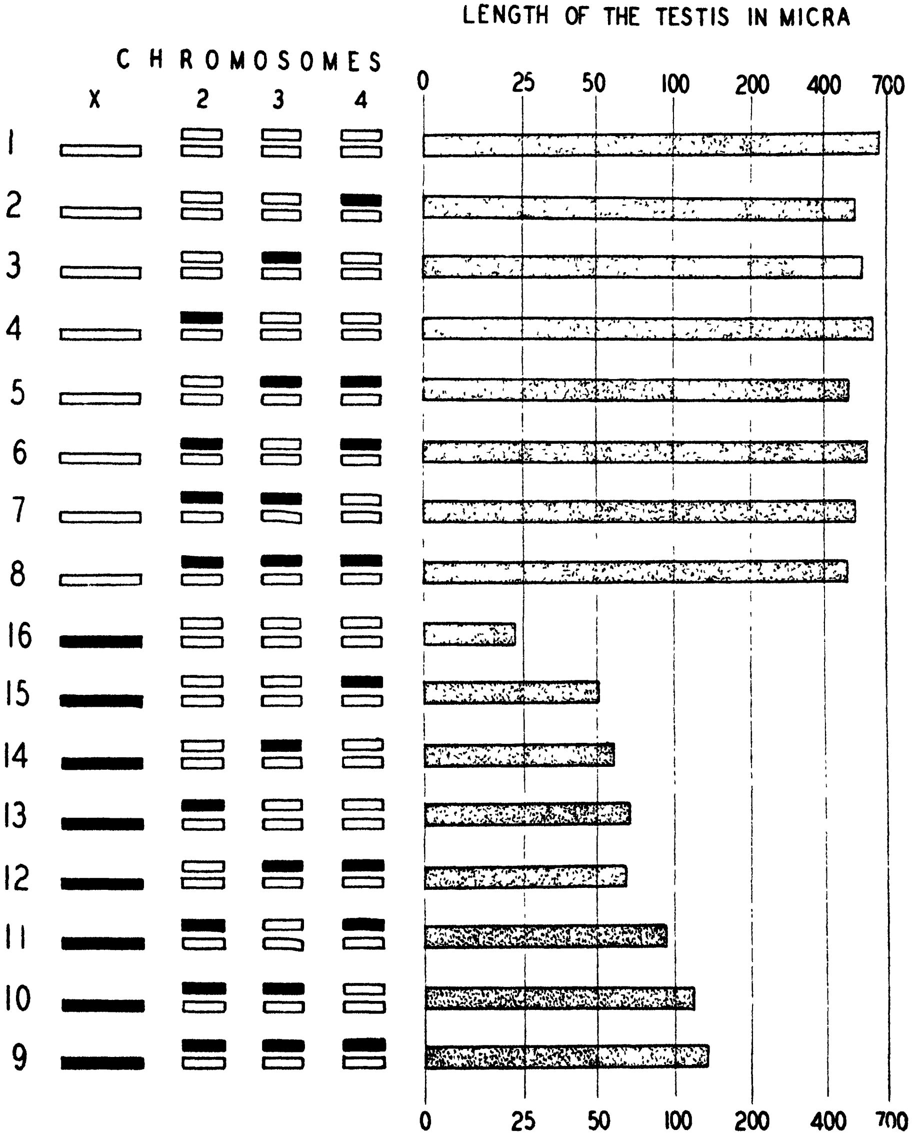

FIGURE 1. The chromosome constitution and the length of the testis in males appearing in the backcross of the F_1 hybrid females to race A males. Race A chromosomes—white; race B chromosomes—black. Only the non-crossover classes are represented.

males of every class have a greater chance to have the chromosomal constitution indicated by the marking genes they carry than any other constitution. In other words, since the conclusions are to be based on averages and not on individual variants, the disturbing influence of the undetected crossovers will manifest itself in a great variability and not in a distortion of the relationships of different classes. These remarks apply to all the experiments described in this article.

Every individual resulting from a backcross to race A male necessarily has one whole complement of autosomes from race A. All males have also a Y chromosome from race A. However, some of them have a race A X chromosome, and others a race B X chromosome. They inherit from their mothers one or more autosomes of race A, or of race B. Not counting crossovers, and disregarding the fifth chromosome, the backcross males should fall into sixteen classes (classes 1–16 in table 1, figure 1).

Males receiving race A X chromosomes (classes 1–8, 17–24) have distinctly larger testes than those receiving race B X's (classes 9–16, 25–32). It follows that the X chromosome carries genes concerned with the sterility. Next, one may notice that different classes of males carrying the same X chromosome vary greatly as to testis size. Classes possessing the B race X chromosome may be taken up first. Among them the largest testes are observed in individuals having one full set of race B autosomes (class 9, also 25 and 26). Making such males homozygous for the race A fourth chromosome decreases testis size (compare classes 9 and 10, 25 and 27, 26 and 28). The differences are not in all cases statistically significant, but they are always in the same direction. Homozygosis for race A third chromosome also decreases testis size, the effect of the third chromosome being stronger than that of the fourth (compare classes 9 and 11). The race A second chromosome produces a still stronger effect in the same direction (compare 9 and 12, 25 and 29, 26 and 30). The third and the fourth chromosomes of race A being homozygous simultaneously produce as much effect as the second alone (12 and 13). Homozygosis for second and fourth, and second and third depresses testis size still further (14 and 15). Finally, males possessing only a race A autosomes have the smallest testes (class 16). The conclusion is warranted that in males carrying race B X chromosome the testes are larger the more B autosomes they carry (although, as stated above, all these males carry one full set of A autosomes).

The mean values for testis size in males having race A X chromosome are based on a smaller number of flies than those discussed above. Nevertheless, it is quite clear that in these males the relations are reversed, namely the testes are the larger the more race A autosomes they carry. The largest testes are observed in males carrying all or most of race A autosomes (classes 1, 17, 18), and the smallest in males having a full set of B autosomes (8, 23, 24). Hence, a general rule may be formulated thus: in the backcross males the testes are larger the more the autosomes agree in their racial origin with the X chromosome, and vice versa. The second, third, and fourth chromosomes are all concerned, but to a different extent: the second chromosome exerts the strongest effect, the third is next, and the fourth last.

Since two of the chromosomes in this experiment contain more than one marking gene each, some crossovers are detected. This makes it possible to determine (a) whether these chromosomes contain more than one locus concerned with testis size, and (b) if this be the case, which part of a given chromosome exerts a stronger effect.

Three genes, *bd*, *y* and *s*, are present in the X chromosome. Crossing over between *bd* and *y* has been observed in our hybrids only once (class 64), although about 30 percent of crossing over takes place between these genes in pure race A (this is due to the inversions in the left limb of the X suppressing crossing over, TAN 1935). On the other hand, crossing over between *y* and *s* is fairly frequent (though less frequent than in pure race A, where these loci are practically independent). Classes carrying the whole X chromosome of race A (*bd y s*, classes 1–8, 17–24) may be compared with those having the right limb, or a part thereof, of race B (*bd y*, classes 33–40, 49–56). If this comparison is made so that classes differing only in the substitution of the right limb of the X are considered (classes 1 and 33, 2 and 34, 17 and 49, 24 and 55, etc.), the conclusion is that such a substitution decreases testis size. This is observed in twelve out of thirteen such comparisons. On the other hand, classes carrying the whole X chromosome of race B (non-*bd*, non-*y*, non-*s*) may be compared with those having the right limb of the X of race A (classes showing *s*, compare 9 and 41, 10 and 42, 25 and 57 etc.). The comparison shows that in this case the substitution of the right limb invariably results in a marked increase of the testis size. The conclusion follows that the right limb, as well as the left limb, of the X chromosome carry genes concerned with sterility.

In order to compare the relative efficacy of the two limbs of the X chromosome, classes carrying the left limb of race A and the right limb of race B must be compared with those having the left limb of B and the right limb of race A. The classes to be compared should, of course, have identical autosomes (compare 35 and 46, 36 and 45, 37 and 44, 38 and 43, 39 and 42, 40 and 41). It can be seen that classes having the left limb of race A have larger testes, and hence the left limb is more important than the right.

An analysis of the third chromosome, which in this experiment is marked by two genes, *or* and *pr*, can be carried through along lines similar to those for the X chromosome (compare classes 1–8 with 17–24, 9–16 with 25–32, and then 17 with 18, 21 with 22, 25 with 26 etc.). Since the effect of the third chromosome as a whole on testis size is much weaker than that of the X chromosome, the analysis of the effect of the former is more difficult. Nevertheless, the majority of the figures indicate that (a) both the part of the third chromosome carrying *or* and that carrying *pr* are concerned with testis size, and (b) that the part containing *pr* exerts a greater effect than that containing *or*.

TABLE 2

Length of the testis (in μ) in the offspring of the cross:
(bd y s Ba Race A ♀ × or Cy Race B ♂)F_1 *♀ × or Race B ♂*

CLASS NO.	PHENOTYPE	M± m	σ	LIMITS	n
1	*or Cy*	475.4± 4.3	60.7	244–661	200
2	*or*	468.8± 4.3	60.0	244–661	200
3	*Cy*	565.3± 3.9	55.2	313–748	200
4	*Ba or Cy*	375.0± 4.5	57.1	174–557	158
5	wild type	561.5± 4.1	59.0	296–730	204
6	*Ba or*	336.5± 4.7	60.9	157–609	168
7	*Ba Cy*	393.8± 5.7	67.0	191–644	138
8	*Ba*	372.2± 4.7	56.7	157–574	144
9	*bd y s Ba*	428.6± 9.2	61.6	278–592	45
10	*bd y s Ba Cy*	325.6±11.7	61.8	139–470	28
11	*bd y s Ba or*	278.4± 8.9	53.2	139–435	36
12	*bd y s*	156.8± 5.9	55.0	70–313	86
13	*bd y s Ba or Cy*	211.4± 5.9	39.8	70–365	46
14	*bd y s Cy*	123.0± 3.7	39.0	35–244	111
15	*bd y s or*	109.8± 3.6	37.8	35–261	108
16	*bd y s or Cy*	81.4± 3.6	35.7	0–157	98
17	*s or Cy*	284.5± 7.9	55.9	139–505	52
18	*s or*	300.0± 6.9	58.8	122–487	73
19	*s Cy*	458.0±10.4	60.0	296–644	33
20	*s Ba or Cy*	257.9±15.5	67.7	104–418	19
21	*s*	468.8± 8.5	59.9	313–661	50
22	*s Ba or*	330.9±13.8	66.5	122–487	23
23	*s Ba Cy*	324.0±14.0	56.0	209–470	16
24	*s Ba*	426.3±14.9	64.9	278–574	19
25	*bd y Ba*	488.4±14.3	53.2	383–644	14
26	*bd y Ba Cy*	453.1±22.1	79.7	244–626	13
27	*bd y Ba or*	313.2		313	2
28	*bd y*	258.6±13.5	63.5	122–435	22
29	*bd y Ba or Cy*	208.8		139–365	8
30	*bd y Cy*	198.5±11.3	37.4	157–313	11
31	*bd y or*	115.4±12.6	48.7	52–313	15
32	*bd y or Cy*	119.2		70–278	8

Backcrosses to race B

Race A females carrying the genes beaded, yellow, short, Bare and purple (*bd y s Ba pr*) were crossed to race B males carrying orange and Curly (*or Cy*). In the F_1 generation females heterozygous for all these genes were selected, and backcrossed to race B males homozygous for orange. The experiment is, then, analogous to that described above, with the exception that since the male to which the hybrid females are backcrossed belongs to race B, all the offspring appearing in the next generation will necessarily carry one complete set of B race (instead of A race as in the preceding experiment) autosomes, and some of them may carry B race chromosomes exclusively. Moreover, the gene purple does not manifest itself in the offspring of the backcross, since the father of the backcross is homozygous for the wild type allelomorph of this gene.

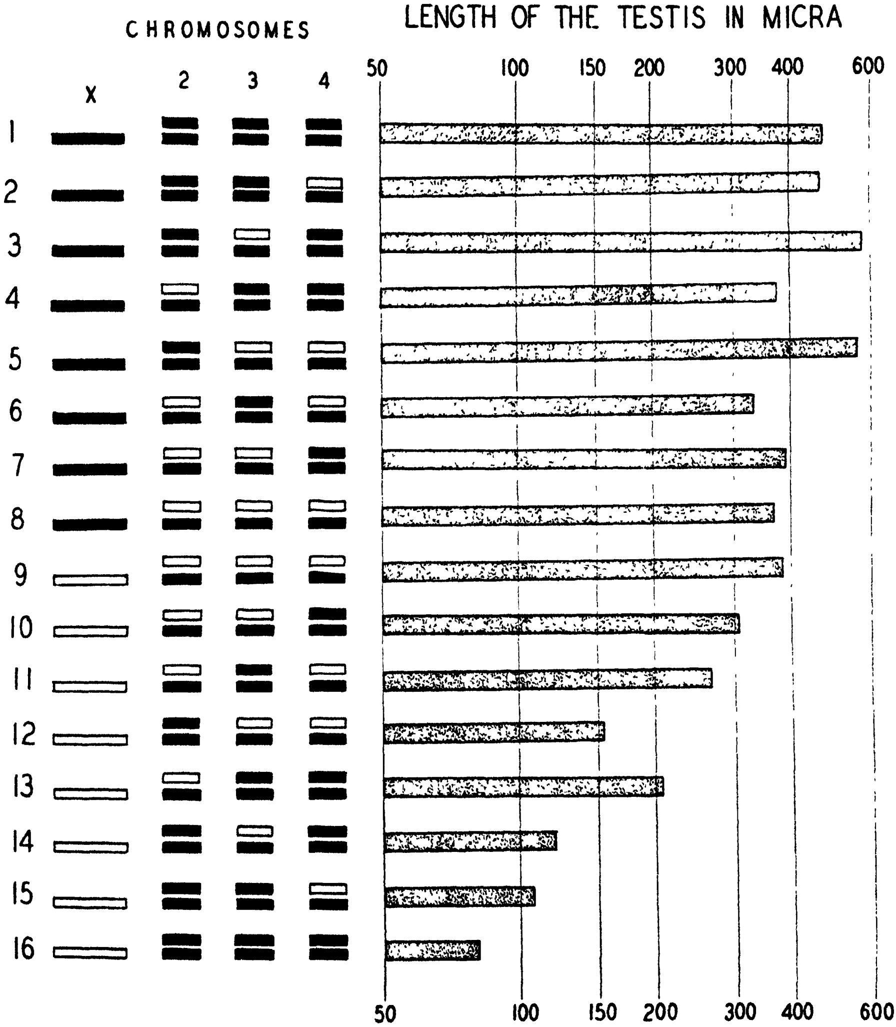

FIGURE 2. The chromosome constitution and the length of the testis in males appearing in the backcross of the F_1 hybrid females to race B males. Race A chromosomes—white; race B chromosomes—black. Only the non-crossover classes are represented. (The bar for class 9 should extend to 428.6; see table 2.)

The measurements of testis size in the males obtained from this backcross are summarized in table 2 and figure 2. Among the classes in which no crossovers are detected (classes 1–16), the first eight (1–8) carry the

X chromosome of race B, and, with one exception, have distinctly larger testes than the classes (9–16) carrying the X chromosome of race A. Thus, with a majority of the autosomes coming from race B, a race B X chromosome is required if large testes are to be formed—a result completely analogous, but reverse in sign, to that arrived at above by studying the backcross to race A males.

Among the males carrying the same X chromosome, the size of the testes depends upon the autosomes. Males receiving an X chromosome of race A (classes 9–16) have the largest testes if they carry one complete set of race A and one set of race B autosomes (class 9). If such males are made homozygous for race B fourth chromosome, the testis size is decreased (compare 9 and 10, 11 and 13, 12 and 14, 15 and 16). A similar, but stronger, effect is produced by homozygosis for the third chromosome of race B (compare 9 and 11, 10 and 13, 12 and 15, 14 and 16), and, finally, the second chromosome exerts the strongest effect (classes 9 and 12, 10 and 14, 11 and 15, 13 and 16). The conclusion is warranted that males having the race A X chromosome have the larger testes the more race A autosomes they carry.

By analogy with the conclusion just stated, as well as with the conclusion arrived at from the study of the backcross to race A, one might expect that in the present experiment males carrying race B X chromosome should have the larger testes the more race B autosomes they carry. Indeed, this is partly, but only partly, true. Among such males, those that are homozygous for race B fourth chromosome have larger testes than those carrying one race A fourth chromosome (compare classes 1 and 2, 3 and 5, 4 and 6, 7 and 8), and those homozygous for race B second chromosome have larger testes than those carrying one race A second chromosome (compare 1 and 4, 2 and 6, 3 and 7, 5 and 8). Turning to the third chromosome, one is at once struck by the fact that it shows a relationship that is the reverse of the expected one: males homozygous for the race B third chromosome have smaller testes than those possessing one third chromosome of race A (compare 1 and 3, 2 and 5, 4 and 7, 6 and 8). This inconsistency is to be discussed below.

Crossing over between *y* and *s* in the X chromosome produces males carrying an X the right limb of which (or a part thereof) comes from one race, and the left limb from the other race. One may observe that males having the right limb of the X chromosome from race A, and the left from race B (showing the effects of the gene *s*, but not of *bd* or *y*, in their phenotype), have mostly smaller testes than those carrying an intact X chromosome of race B (compare classes 17–24 with 1–8). Conversely, males carrying the right limb of the X from race B and the left from race A (showing *bd* and *y*, but not *s*), have larger testes than males with complete race A X chromosome (compare 25–32 with 9–16). Finally, with the autosomes held constant, males having the right limb of the X from race A and the left limb from race B have mostly larger testes than males carrying the right limb of the X from B and the left from A (compare classes 17 and 32, 18 and 31, 19 and 30, etc.). It follows from this that (a) both limbs of the X chromosome carry sterility genes, and that (b) the sterility genes in the left limb are either more numerous or more effective than those in the right limb. Both conclusions are indentical with those reached from the study of the backcross to race A.

Returning again to the unexpected behavior of the third chromosome in males carrying the X chromosome of race B (see above), one might notice that in the experiment under consideration the cross was so arranged that all the hybrids originated from race A females (*bd y s Ba pr*), and, consequently, have race A cytoplasm. This fact may arouse the suspicion that homozygosis for the race B third chromosome is incongruent with race A cytoplasm, in such a way that in males carrying race A cytoplasm and all or a majority of race B chromosomes, the presence of one race A third chromosome may increase, instead of decrease, the testis size. If this were true, we would have the first indication that the cytoplasms of races A and B are specifically distinct and may be concerned with the production of the sterility of the hybrids. The possibility just stated can be subjected to a rigorous experimental test: a cross must be arranged which should produce offspring completely comparable with those from the cross just discussed in the chromosomal constitution, but which should have throughout the cytoplasm of race B, instead of that of race A. This requirement is fulfilled in the following experiment.

Race B females carrying *or* and *Cy* were crossed to *bd y s Ba pr* males of race A. F_1 females heterozygous for all these genes were backcrossed to race B males homozygous for *or*. All the strains used in this cross were the same as those used in the preceding experiments. The results are summarized in table 3. They should be compared to those presented in table 2. In these tables, the classes showing identical phenotypes have similar chromosomal constitutions, but differ in that the data of table 2 pertain to flies having race A cytoplasm, and those of table 3 to flies having race B cytoplasm. It is easily noticeable that the corresponding classes of males shown in table 2 have consistently larger testes than those shown in table 3. However, this fact is probably of no particular significance, since the experiments on which tables 2 and 3 are based were done at different times, and, due to the extreme sensitivity of the testis size to environmental conditions, no two separate experiments are likely to agree as far as the absolute size of the testes of hybrid males is concerned. Far more consequential is the relative testis size in males of different classes in each

TABLE 3

Length of the testis (in μ) in the offspring of the cross:
(or Cy Race B ♀ × bd y s Ba Race A ♂)F_1 ♀ × or Race B

CLASS NO.	PHENOTYPE	M ± m	σ	LIMITS	n
1	*or Cy*	401.6 ± 7.2	72.9	226-609	103
2	*or*	404.9 ± 6.5	68.0	261-557	109
3	*Cy*	541.7 ± 7.4	80.6	313-731	118
4	*Ba or Cy*	298.6 ± 6.4	77.6	122-470	146
5	wild type	518.0 ± 6.6	71.9	348-679	120
6	*Ba or*	296.5 ± 6.3	72.4	139-487	133
7	*Ba Cy*	342.6 ± 7.7	82.7	157-522	114
8	*Ba*	359.1 ± 8.3	89.6	191-557	116
9	*bd y s Ba*	283.4 ± 10.3	63.2	174-452	38
10	*bd y s Ba Cy*	273.9 ± 9.6	64.6	174-400	45
11	*bd y s Ba or*	251.8 ± 7.8	62.1	139-383	63
12	*bd y s*	108.4 ± 3.2	41.9	35-261	171
13	*bd y s Ba or Cy*	194.5 ± 7.3	61.9	70-331	71
14	*bd y s Cy*	79.9 ± 2.5	31.5	35-157	164
15	*bd y s or*	57.8 ± 2.6	31.5	17-139	149
16	*bd y s or Cy*	41.6 ± 2.5	32.7	0-139	177
17	*s or Cy*	213.7 ± 9.9	66.5	70-348	45
18	*s or*	206.4 ± 8.6	57.8	104-348	45
19	*s Cy*	330.1 ± 10.7	66.1	191-505	38
20	*s Ba or Cy*	179.7 ± 14.6	70.1	104-400	23
21	*s*	290.1 ± 11.8	67.7	174-417	35
22	*s Ba or*	195.4 ± 8.8	47.9	104-331	30
23	*s Ba Cy*	313.2		261-418	7
24	*s Ba*	252.3 ± 14.7	62.1	174-417	18
25	*bd y Ba*	464.1 ± 36.7	127.4	278-679	12
26	*bd y Ba Cy*	461.1 ± 22.5	74.1	278-557	11
27	*bd y Ba or*	438.0 ± 18.4	71.5	313-557	15
28	*bd y*	241.3 ± 10.4	67.9	122-418	43
29	*bd y Ba or Cy*	402.6 ± 13.3	44.0	331-487	11
30	*bd y Cy*	194.9 ± 8.9	56.4	87-365	40
31	*bd y or*	140.4 ± 7.7	49.9	52-261	42
32	*bd y or Cy*	122.0 ± 6.5	40.7	70-244	39
33	*y s*	643.8		626-661	2
34	*bd*	87.0		87	1
35	*bd Cy*	417.6		417.6	1
36	*y s or Cy*	556.8		556.8	1

experiment. Approaching the data from the standpoint of this criterion, one is forced to the conclusion that the data of tables 2 and 3 are identical in all essentials. For our purposes, the most important fact is that in males having race B cytoplasm and race B X chromosome, homozygosis for the race B third chromosome produces a decrease, instead of an increase, of the testis size. Hence, the anomalous behavior of the third chromosome in these crosses cannot be due to an interaction between this chromosome and the cytoplasm. No indication of the existence of an inherent difference between the cytoplasms of the two races is apparent.

Obviously, an explanation of the anomalous effect of the third chromosome in the crosses under consideration is to be looked for elsewhere. Two such explanations may be suggested. First, one may suppose that it is a specific property of the third chromosome of race A to increase the testis size in males having a majority of the chromosomes of race B. In this case, a male of hybrid ancestry having one race A third chromosome and the rest of the chromosomes of race B would always have larger testes than pure race B males. Second, the above behavior of the third chromosome may be due to a maternal effect, being manifested only in flies coming from the eggs of a hybrid mother. This amounts to assuming that the presence of the hybrid karyotype in the egg cell, before it has undergone the processes of maturation and fertilization, leaves an impression on this cell that lasts for at least one generation.

The two alternative explanations just suggested are obviously *ad hoc* hypotheses, and should be considered objectionable on this ground, were it not for the fact that they may be tested experimentally. Males of class 3 (table 3) have all the chromosomes of race B except a single race A third chromosome. Their race B third chromosome carries the marking gene *or*. Having large testes, these males are fertile, and can be crossed to pure B females homozygous for *or*. In the offspring of this cross two classes of males must appear. One of them, phenotypically orange, will have only race B chromosomes, and will be genotypically identical with males of classes 1 and 2 in table 3. The other class will be wild type in phenotype, and will be genotypically identical with class 3 in table 3, that is, will carry one race A third chromosome. Now, if the third chromosome of race A has *per se* the property of increasing testis size in males that are otherwise race B in constitution, the wild type males in this experiment should have larger testes than the orange males. Thus, the relationships observed in the first backcross generation (tables 2 and 3) should be repeated in the next generation. If, on the other hand, the phenomenon under consideration is due to a maternal effect, the testes of the wild type and the orange males should be either equal in size, or else the orange males should have larger testes than the wild type ones.

The experiment has been arranged as just outlined, and the testes in the resulting males were measured. Their size (in μ) was:

orange	607.6 ± 8 6
wild type	578.6 ± 7.4

The difference between these figures is not statistically significant, but the orange males have testes either equal to or larger than the wild type ones. The maternal effect hypothesis is correct. Maternal effects have been observed in *Drosophila pseudoobscura* crosses more than once (Dobzhansky 1935, Dobzhansky and Sturtevant 1935).

STERILITY GENES IN THE SECOND CHROMOSOME

The experiments reported above show that genes responsible for the sterility of the interracial hybrids are located in the X, second, third, and fourth chromosomes—all chromosomes except the fifth, which has not been followed in the crosses. Furthermore, it has been shown that in the X and also in the third chromosome more than one sterility gene is present, located in different parts of the respective chromosomes. The question whether the second chromosome has one or more sterility genes could not be answered, since this chromosome carried a single gene marker, namely Bare. In the experiment now to be discussed this drawback is removed by introducing two marking genes in the second chromosome.

It may also be noticed that in the former experiments a majority of the marking genes were introduced through the race A parent, causing the classes of the offspring having mainly A race chromosomes to appear in relatively low frequencies. In the following experiment mainly race B markers are used.

Race B females carrying the sex-linked recessives scutelar (*sc*) and dela (se^d), and the second chromosome recessive cinnabar (*cn*) and dominant Smoky (*Sm*), were crossed to race A males carrying the fourth chromosome dominant Curly (*Cy*). The F_1 females heterozygous for these genes were backcrossed to race B cinnabar males. The results are summarized in table 4. The gene Curly involved in this cross was originally obtained as a mutant in race B, and "transferred" into race A by means of repeated backcrosses of Curly flies to race A males (see above). Thus, the race A fourth chromosome marked by *Cy* in this experiment is really a composite chromosome, containing, presumably, most of the material from race A, and a more or less small section including the locus of the gene *Cy* from race B.

To start with, one may notice that the data of table 4 corroborate the general conclusions regarding the action of the sterility genes previously discussed. Since males recorded in table 4 have at least one full set of race B autosomes, the classes carrying an intact race B X chromosome (1–4, 17–20) have larger testes than those carrying an intact X of race A (5–8, 21–24). Among males carrying the same X chromosome, largest testes are present in those that have most autosomes of the same race as the X chromosome (class 1—all chromosomes of race B, class 5—race A X chromosome and one set of A autosomes), and smallest testes in males having an X of one race and the autosomes of the other (class 4 with X of race B and one set of race A autosomes, class 8 with X of race A and race B autosomes). The fourth chromosome marked by *Cy* behaves as a race A chromosome in spite of the fact that it carries a section coming from race B. Crossing over in the X chromosome leads to the results expected on the basis of the previously reported experiments: males having the left part of the X from race B (carrying *sc*) have larger testes than males having the right part of the X from race B but otherwise similar (males showing *se* but not *sc*).

TABLE 4

Length of the testis (in μ) in the offspring of the cross:
(sc se cn Sm Race B ♀ ×Cy Race A ♂)F_1 ♀ ×cn Race B ♂.

CLASS NO.	PHENOTYPE	M± m	σ	LIMITS	n
1	*sc se cn Sm*	563.6± 6.7	56.6	418-679	103
2	*sc se cn Sm Cy*	523.2±10.3	76.9	400-714	72
3	*sc se*	449.6±10.6	84.4	278-626	61
4	*sc se Cy*	420.7± 9.3	70.1	261-592	56
5	*Cy*	319.5± 7.2	76.2	122-522	114
6	wild type	282.6± 6.1	67.0	122-435	122
7	*cn Sm Cy*	97.1± 4.0	36.9	35-174	85
8	*cn Sm*	90.7± 4.4	40.9	17-209	87
9	*sc cn Sm*	455.9±10.0	58.3	365-592	34
10	*sc cn Sm Cy*	416.6±12.5	70.5	226-592	32
11	*sc*	426.0±10.0	74.1	261-592	55
12	*sc Cy*	435.4± 8.8	68.2	296-592	60
13	*se Cy*	433.3±10.4	66.1	278-557	40
14	*se*	420.0±11.7	78.0	278-592	45
15	*se cn Sm Cy*	120.6± 9.7	51.7	52-244	28
16	*se cn Sm*	86.0± 5.7	32.2	0-174	32
17	*sc se cn*	578.6± 9.2	63.3	452-661	48
18	*sc se Sm*	371.0± 9.7	60.9	209-487	39
19	*sc se cn Cy*	554.0±12.6	78.7	388-696	39
20	*sc se Sm Cy*	364.2±10 5	55.7	226-452	28
21	*Sm*	241.2± 9.9	74.1	87-452	56
22	*cn*	99.0± 4.5	33.1	52-243	54
23	*Sm Cy*	248.8± 9.0	69.4	104-418	60
24	*cn Cy*	121.3± 7.5	46.1	0-226	28
25	*sc cn*	492.9±11.2	66.1	348-626	35
26	*sc Sm*	407.5±11.8	60.4	313-557	26
27	*sc cn Cy*	500.6±18.6	86.8	261-609	22
28	*sc Sm Cy*	396.5±11.9	63.2	296-505	28
29	*se cn*	102.3± 7.8	39.8	52-191	26
30	*se Sm*	401.4±12.8	61.6	226-487	23
31	*se cn Cy*	116.8± 8.8	43.0	70-261	28
32	*se Sm Cy*	463.9±18.3	79.3	296-626	19

The detected crossing over in the second chromosome gives rise to males showing *Sm* but not *cn*, and *cn* but not *Sm* (classes 17-32). Males carrying the X and the second chromosomes of race A (classes 5 and 6) have larger testes than the corresponding classes of males in which a part of the second chromosome of race B is present (classes 21-24), but the latter have larger testes than males carrying the X of race A and the whole second chromosome of race B (classes 7-8). Males possessing the X and the second chromosomes of race B (classes 1 and 2) have testes equal to or larger than similar males carrying a part of the second chromosome of race A (classes 17–20). In either case, the substitution of the right part of the second chromosome (carrying *Sm*) produces less effect than the substitution of the left part of the second chromosome (carrying *cn*).

The conclusion is warranted that more than one locus concerned with sterility is present in the second chromosome, and that the part of the chromosome marked by *cn* carries either more numerous or stronger sterility factors than the part marked by *Sm*. This result was not unexpected, in view of the fact that, according to the data of STURTEVANT, the gene *Sm* is easily transferred from race B to race A, whereas we have not been able to transfer *Ba* (lying very close to *cn*) from race A to race B. A less extensive series of attempts to transfer *cn* from race B to A was also unsuccessful.

Since the real position of the genes *cn*, *Ba*, and *Sm* in the second chromosome (in terms of the cytological map) is as yet unknown, it is not possible to decide which of the two parts of the chromosome is longer, and hence it remains obscure whether or not the effectiveness of a given chromosome section is proportional to its cytological length.

FERTILITY TESTS

The main body of our conclusions regarding the localization of the sterility genes is based on testes measurements, not on direct fertility tests. The justification of the procedure is given above, and need not be repeated here. Some fertility tests were performed as an additional check.

In the offspring of the backcross to race B the results of which are represented in table 2 males of the classes 1–8 were selected and crossed to pure race B females. Fifty males of each class were segregated into batches of five, and each batch was placed with 3–4 females in a separate culture bottle. No tests of individual males were made, since the males appearing in the backcrosses are, in contradistinction to males of pure races or the F_1 hybrids, rather weak, sometimes somatically abnormal, and in general inferior in vigor. The causation of this decrease in vigor in backcross males constitutes a separate problem (DOBZHANSKY and STURTEVANT 1935).

Males of the classes 5, 6, 7 and 8 produced no offspring. Hence, males carrying a second chromosome of race B and an X of race A are always sterile (which was to be expected since they have testes distinctly smaller than normal). All culture bottles containing males of the classes 1, 2, 3, and 4 produced offspring, although some of them did so only after repeated transfers on fresh food, and even then the offspring were few in number. The conclusion follows that males carrying all race B chromosomes in race A cytoplasm may be fertile, and the presence of the race A third or fourth chromosomes, or both together, with race B X and second chromosomes, does not necessarily prevent fertility.

THE SECOND BACKCROSS GENERATION

Females appearing in the first backcross generation fall into as many distinct genotypic classes as do their brothers. All of them possess a full set of the chromosomes of the race to which their father belonged, but the second set of the chromosomes inherited from the mother may be of either race, or may be partly derived from one race and partly from the other.

Some females that were sisters of the males whose testis measurements are presented in table 2 (coming from a backcross of the F_1 hybrid females to race B males) were individually crossed to race B males homozygous for orange. Since these females have their race A chromosomes marked by mutant genes, their own genetic constitution, as well as the genetic constitution of their male offspring, can be judged by the phenotype. No testis measurements were made on the males of the second backcross generation, but the testis size was evaluated by dissection and a simple inspection.

The results obtained are summarized below.

1. Females carrying all chromosomes of race B (*or Cy* in phenotype). Four such females tested; all sons have testes of normal size.

2. Females carrying an X of race A, rest of the chromosomes race B (*or Cy* in phenotype). One female tested; wild type sons have large testes, *bd y s* sons small to intermediate.

3. Females carrying a race A fourth chromosome, rest of the chromosomes race B (*or* in phenotype). Two tested; all sons have large testes.

4. Females carrying a race A third chromosome, rest of the chromosomes race B (*Cy* in phenotype). Five tested; four produced all sons with large testes, one gave sons with testis size varying from large to intermediate.

5. Females as above, but also one race A X chromosome (*Cy* in phenotype). Two tested; *bd y s* sons have small testes, wild type ones large testes.

6. Females carrying one third and one fourth chromosome of race A, the rest of the chromosomes of race B (wild type in phenotype). Seven tested; two produced sons with large testes, and five gave sons with testes varying from large to intermediate.

7. Females as above, but also one race A X chromosome (wild type in phenotype). One tested; *bd y s* sons had very small testes, non-*y* sons large to intermediate.

8. Females having one second and one fourth chromosomes of race A, the rest of race B (phenotype *Ba or*). One tested; *Ba* sons with very small testes, non-*Ba* sons with testes of intermediate size.

9. Females having one second and one third chromosomes of race A, the rest of race B (phenotype *Ba Cy*). Four tested; wild type and *Cy* sons had

testes of intermediate size, *Ba Cy* sons—small testes, *Ba* sons—very small testes.

10. Females having one set of autosomes of race A, the X's and the other set of autosomes of race B (phenotype *Ba*). One tested; wild type sons had intermediate or small testes, *Ba* sons very small testes.

These data, meager as they are, corroborate the conclusions regarding the distribution of the sterility factors in the chromosomes reached in the main body of the work. The failure to test a large number of the backcross females is due to the fact that they are on the whole much weaker than the F_1 hybrid females, and frequently produce no offspring.

DISCUSSION

The possibility that the sterility of the interracial hybrids in *Drosophila pseudoobscura* is due to an accumulation of structural differences between the chromosomes of the two races may be considered excluded. Some arguments against this possibility were presented in an earlier publication (DOBZHANSKY 1934). More recently TAN (1935a, b) has studied the chromosomes of the salivary gland cells in both races, and has found that they differ in six inverted sections, four of which are located in the X chromosome, one in the second and one in the third. Since in *D. pseudoobscura* the sterility is confined to the male hybrids, only the two autosomal inversions come under consideration as a possible cause of sterility. *D. melanogaster* individuals of either sex heterozygous for five inversions (delta-49 in the X chromosome, CIIL and CIIR of Curly in the second, CIIIL and CIIIR of Deformed in the third chromosome) are fertile. According to the unpublished data of STURTEVANT, the wild strains of *D. pseudoobscura* found in nature are frequently heterozygous for inverted sections in the third, and less frequently also in the second and in the X chromosomes, but no sterility is apparent in these strains. The autosomal interracial inversions discovered by TAN are remarkable neither in their cytological length nor in the extent of the suppression of crossing over they produce in the chromosomes concerned. The fourth chromosomes of the two races are, according to TAN, similar in gene alignment, but a failure of their pairing is frequently observed in the interracial hybrids. In short, the evidence against these inversions directly causing the sterility in *D. pseudoobscura* appears conclusive.

The data presented in this article show that genetic factors causing sterility of the interracial hybrids exist in all the chromosomes tested, that is in all the chromosomes except the fifth. Moreover, in the X, the second, and the third chromosomes respectively more than one sterility factor was detected. On the other hand, the effects of the different chromosomes are not equally strong: the left limb of the X has the strongest effect, the right limb of the X, the second, the third, and the fourth chromosomes are decreasingly effective in the order indicated. This fact may be interpreted as indicating that "main sterility factors" lie in the X and in the second chromosomes, while the third and the fourth contain minor contributing factors or modifiers. This interpretation is, however, not a necessary one. The cytological lengths of the chromosomes (in salivary gland cells, according to TAN) decrease in the following order: X chromosome, second, third, and fourth chromosomes. Hence, the effectiveness of a chromosome in producing sterility is on the whole proportional to its length. That no strict proportionality of this sort obtains is clearly demonstrated by the greater effectiveness of the shorter left limb of the X as compared with the cytologically longer right limb of the same chromosome, but the available data permit no classification of the sterility factors into main and modifying ones. The greater effectiveness of the part of the second chromosome carrying the loci of Bare and cinnabar as compared with the part of the same chromosome carrying Smoky also gives no evidence on this point since the cytological lengths of these parts are as yet unknown. Likewise, the question of whether or not the parts of the chromosomes carrying interracial inversions are especially likely to contain numerous or powerful sterility genes remains open.

The available data are best interpreted as meaning that the testis size in the backcross hybrids is the larger the more their X chromosome agrees with the autosomes as to its racial origin. In other words, sterility versus fertility seems to be determined by interactions of factors located in the X chromosome with factors located in the autosomes. Here again, this interpretation is not a necessary one, since the cytological length of the X of *D. pseudoobscura* is almost equal to the sum of the lengths of all the autosomes. Hence, the data are not inconsistent with the supposition that testes are smallest in males carrying equal volumes of the chromosomal material from both races, and that the more homogeneous are the chromosomes in their racial origin, the larger are the testes.

Concerning the mechanism of the action of the sterility factors, the available data permit a single conclusion only, namely that their effects on testis size are additive. This is amply demonstrated by the figures in tables 1–4: if two chromosomes, or sections of chromosomes, each produce a decrease (or an increase) in testis size, these two chromosomes, or sections, produce a larger decrease (or increase) if they are present simultaneously. More complicated forms of interactions, for instance factors whose effects are contingent on the presence of other factors, have not been detected. The only exception is the third chromosome of race B, the action of which in the hybrids possessing predominantly race B chromosomes (or at least the race B X chromosome) is complicated by a maternal effect (see the discussion of this point in the text). It is of interest that in hybrids carrying predominantly race A chromosomes the third chromosome does not show any anomalous effects.

SUMMARY

1. Crosses between race A and race B of *Drosophila pseudoobscura* produce in F_1 fertile daughters and sterile sons. The F_1 females may be backcrossed to males of either parental race. Some of the males in the resulting offspring are fertile and others are sterile. Fertile males always have large testes, while testes in the sterile males vary in size from normal to very small. The testis size is an index of the degree of disturbance in the process of spermatogenesis, the disturbance being greatest in the smallest testes.

2. Backcross males having only race A chromosomes are fertile irrespective of whether they have the cytoplasm derived from race A or from race B. Backcross males having only race B chromosomes are likewise fertile irrespective of the source of their cytoplasm.

3. In the offspring of the backcross of the F_1 hybrid females to race A males, the males carrying the X chromosome from race A have larger testes than those carrying the X chromosome from race B (table 1, figure 1). Among classes carrying the same X chromosome testis size depends upon the autosomes: the more the autosomes agree in their racial origin with the X chromosome the larger are the testes, and vice versa.

4. Backcrosses of the F_1 hybrid females to race B males give results analogous to the above but reverse in sign: the largest testes are present in backcross males having the race B X chromosome and race B autosomes, and smallest testes in males carrying race A X chromosome and B race autosomes (tables 2, 3, and 4, figure 2).

5. All the chromosomes studied carry genes concerned with testis size, and consequently with sterility. The X chromosome produces, however, the strongest effect, the second chromosome follows next, and the third and the fourth chromosomes last. Thus, on the whole, the effectiveness of each of the chromosomes is proportional to its cytological length.

6. Wherever in our experiments a chromosome was marked by more than one mutant gene it was possible to show that sterility genes are present in different parts of this chromosome.

7. The effects of the sterility genes located in the different chromosomes and sections of the chromosomes are additive.

8. The behavior of the third chromosome of race B in the hybrid males of the first backcross generation possessing a majority of race B chromosomes is anomalous. Such males have larger testes if they carry one race A and one race B third chromosome than if they are homozygous for the race B third chromosome. This anomaly is due to a maternal effect: in in-

dividuals coming from the eggs of the F_1 hybrid females homozygosis for the race B third chromosome decreases the testis size.

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SEX REVERSAL IN APLOCHEILUS LATIPES AND A NEW EXPLANATION OF SEX DIFFERENTIATION

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INTRODUCTION

IN *Aplocheilus latipes* where the male is digametic XY two allelomorphic genes concerning the body color, orange-red (*R*) and white (*r*) are located in the X and Y chromosomes (AIDA 1921). Heterozygous orange-red males X*R*Y*r* in crosses with white females X*r*X*r* produce orange-red females X*R*X*r* and white males X*r*Y*r* in criss-cross manner, though sometimes a few exceptional orange-red males may be found. Some of these abnormal males produce in crosses with normal females offspring with an abnormal sex ratio, the females always predominating in number and the males which are all abnormal like their male parent being very few or not present at all. By carrying on the breeding of these males generation after generation I found that the proportion of males gradually increases (AIDA 1930), and I tried in the former report to explain these abnormal males as the product of non-disjunction in sex chromosomes having XXY constitution.

WINGE (1930) found in *Lebistes reticulatus* sex-reversed males of female genotype XX which in crosses with normal females produced female offspring only. On account of the similarity of breeding results between his observation on sex reversal and ours on abnormal males he suggested that our male fish are also produced by sex reversal, but not by non-disjunction.

To determine which one of these alternative conceptions is more valid further research has been continued. A new fact irreconcilable with the supposition of non-disjunction was found, and at present I am inclined to believe that our males are, as WINGE has assumed, sex-reversed males of the female genotype XX. Moreover I was able to produce at the same time females which are unquestionably due to sex reversal, viz. females of male genotype XY.

The possibility of sex alteration in so well differentiated a gonochorist as Aplocheilus and the breeding results induced me to make a new hypothesis on sex differentiation in our fish.

SEX-REVERSED MALE OF FEMALE GENOTYPE

1. *Effect of individuality of male parent on sex differentiation*

Among a great number of matings of abnormal males in 1927 (table 4, Aida 1930) the male designated as K10,8 produced male offspring in the highest ratio, viz. 4 ♀ : 1 ♂, and male M2 also in pretty high ratio, viz. 10 ♀ : 1 ♂. In 1928 some male offspring from these two matings were each mated to five females from the same litter as the male.

Table 1

Male offspring of the males K10, 8 and M2 crossed each with 5 females from the same litter as the male.
R = Orange-red. r = White.

NO. AND YEAR OF MATING	PARENTS		OFFSPRING				TOTAL		RATIO
	♂	♀	♀♀R	♀♀r	♂♂R	♂♂r	♀♀	♂♂	♀ : ♂
B 26 '28	R K10, 8, 3	R 5	190	71	27	15	261	42	6
B.27 '28	R K10, 8, 2	R 5	145	68	27	27	213	54	4
B.28 '28	R K10, 8, 4	R 5	149	44	6	1	193	7	28
B.30 '28	R K10, 8, 5	R 5	61	27	3	0	88	3	29
B.31 '28	R K10, 8, 6	r 5	104	83	8	5	187	13	14
B 32 '28	R K10, 8, 7	r 5	92	78	8	4	170	12	14
B.16 '28	R M2, 4	r 5	47	17	3	1	64	4	16
B.17 '28	R M2, 6	r 5	291	284	50	46	575	96	6
B.18 '28	R M2, 2	r 5	37	53	5	5	90	10	9
B.19 '28	R M2, 8	r 5	324	343	62	68	667	130	5
B.20 '28	R M2, 7	r 5	80	79	9	7	159	16	10

In the above tabulated matings the ratio of males to females varies from 4 to 29, and no constancy is found notwithstanding the fact that the parents of each mating were taken from the same litter.

All matings were carried on at the same season of the year under similar conditions as possible, so that the difference of external influences is excluded. As in each mating the five females, all taken from the same litter, were crossed with the single male, if some different tendency in producing the male number should exist in the female parent, it might have been mitigated, and would not affect so much the ratio of male number in offspring as we have seen. The actual variation of the ratio, accordingly, may be considered to depend on the nature of the male parent.

2. *Effect of individuality of female parent on sex differentiation*

In the next year (1929) a single male was mated simultaneously to two females in the same aquarium, each from different litters, and the sex numbers were counted in the offspring of each female parent separately.

TABLE 2

Three orange-red males, A, B, and C, from mating 26, 1928, were each mated to single orange-red female from the same litter and also simultaneously to single white female from mating 29, 1928, where only females and no males were produced 170 ♀ ♀, 0 ♂.

R = Orange-red. r = White.

NO. AND YEAR OF MATING	PARENTS		OFFSPRING				TOTAL		RATIO
	♂	♀	♀♀*R*	♀♀*r*	♂♂*R*	♂♂*r*	♀♀	♂♂	♀·♂
B.9 '29	A	*R* from B.26, '28	62	24	33	12	86	45	2
B.12 '29		*r* from B.29, '28	38	30	31	29	68	60	1
B.10 '29	B	*R* from B.26, '28	97		21		97	21	5
B.13 '29		*r* from B.29, '28	136	1			137	0	No males
B.11 '29	C	*R* from B.26, '28	77	18	33	13	95	46	2
B.14 '29		*r* from B.29, '28	69	59	6	1	128	7	18

The breeding results in the table 2 show clearly that the same male may produce in matings to different females male offspring in different ratios. That this variation of the ratio is caused by the difference of the individual tendencies of female parents is to be conjectured.

When the females are taken from the wild stock and bred to any abnormal male, the offspring are always all female with none or very few males. Even the males which in crosses with colored females produce many male offspring yield in simultaneous crosses with wild females female offspring only. The breeding results of the same male mated to the colored and wild females at the same time in the same aquarium are summarized in the next table.

TABLE 3

Abnormal male crossed with colored and wild females.

BR = Brown, the wild color. *B'R* = Orange-red variegated with black. *B'r* = White variegated with black. *R* = Orange-red. *r* = White.

NO. AND YEAR OF MATING	PARENTS ♂	PARENTS ♀	OFFSPRING ♀♀	OFFSPRING ♂♂	TOTAL ♀♀	TOTAL ♂♂	RATIO ♀ : ♂
B.25 '32	*B'r* (B 20, '31)	*R* (B.20,'31)	*B'R* 16 *B'r* 16 *R* 7 *r* 10	*B'R* 16 *B'r* 17 *R* 12 *r* 13	49	58	1
B.34 '32		*BR* (Wild)	*BR* 120	0	120	0	
B.1 '33	*R* (B 28, '32)	*r* (B 28,'32)	*R* 74 *r* 81	*R* 19 *r* 22	155	41	4
B 2 '33		*BR* (Wild)	*BR* 153	*BR* 0	153	0	
B 15 '33	*B'R* (B.26, '32)	*r* (B.26,'32)	*B'R* 29 *B'r* 29 *R* 23 *r* 22	*B'R* 0 *B'r* 3 *R* 6 *r* 3	103	12	9
B.16 '33		*BR* (Wild)	*BR* 310	0	310	0	

Table 3 shows that the very same males which have produced in crosses with colored females males in the ratio of one-fifth, one-tenth or even more than one-half of the total offspring, yielded in mating to wild females, females only and no males at all. The wild females, nevertheless, do not produce constantly females only, thus sometimes when the number of the offspring reared is large enough a few males may be found. For instance, in mating 2, 1931, where an abnormal male was mated to three wild females, only one male was found among 719 offspring, the remaining ones being females.

From these results the inference may be drawn that the females of our colored varieties have some tendency to produce a greater number of male offspring than the wild normal females.

3. *Seasonal influence on sex differentiation*

The ratio of males produced varies according to the different seasons. Our fish spawn every morning throughout the whole warm season, and the sex ratio in the offspring from the same parents differs according as they are hatched out in early or mid-summer. In the next table the numbers of male and female offspring from the same parents reared in each period are separately listed. The water temperature at the surface in the morning before sunrise was in the first period 23–26°C. and in the second 25–28°C.

TABLE 4

Offspring of the same parents hatched out in earlier and later periods of summer.

BR, *B'R*, *B'r*, *R'r* same as in former table. *Br* = Blue.

NO. AND YEAR OF MATING	PARENTS		HATCHED OUT PERIODS	OFFSPRING								TOTAL		RATIO
	♂	♀		♀♀				♂♂				♀♀	♂♂	♀ : ♂
B.6 ’29	*R* (B.31,’28)	*R*	From June 26 to July 20	*R*	54	*r*	20	*R*	0	*r*	1	74	1	70
			From July 21 to August 10	*R*	52	*r*	21	*R*	18	*r*	7	73	25	3
B.7 ’29	*R* (B.31,’28)	*R*	From June 26 to July 20	*R*	38	*r*	15	*R*	9	*r*	2	53	11	5
			From July 21 to August 16	*R*	16	*r*	6	*R*	17	*r*	7	22	24	1
B.8 ’29	*R* (B.31,’28)	*R*	From June 26 to July 23	*R*	58	*r*	24	*R*	4	*r*	1	82	5	16
			From July 24 to August 17	*R*	40	*r*	20	*R*	30	*r*	10	60	40	1.5
B.23 ’32	*BR* (B.2, ’31)	*r* From normal stock	From July 4 to July 20	*BR* *R*	11 10	*Br* *r*	9 9	*BR* *R*	9 1	*Br* *r*	8 6	39	24	2
			From July 25 to August 6	*Br* *R*	11 17	*Br* *r*	11 12	*BR* *R*	1 1	*Br* *r*	4 3	51	9	6
B.27 ’32	*B'R* (B.16,’31)	*r*	From July 10 to July 20	*B'R* *R*	9 7	*B'r* *r*	9 5	*B'R* *R*	2 0	*B'r* *r*	1 0	30	3	10
			From July 24 to August 6	*B'R* *R*	24 26	*B'r* *r*	10 11	*B'R* *R*	8 5	*B'r* *r*	9 8	71	30	2

From the table we can see clearly that the season has a certain influence in determining the ratio of male offspring. In four matings among five the increase of the male number is very large in the second period. Probably high temperature may induce some females to male reversal. WINGE (1934) reports similar seasonal influence on the sex ratio in offspring of sex-reversed males of Lebistes. Thus he found that though in the early summer a relatively large number of males is produced, in other seasons the number of females increases, the females alone being produced in winter. According to Witschi (1929) female tadpoles of *Rana sylvatica* reverse to males at high temperature. Thus the temperature seems to have a similar effect on sex differentiation in lower vertebrates.

4. *Excess of male number*

The mating 9, 1930, yielded male offspring in a high ratio, viz., 140 ♀ ♀ and 81 ♂ ♂. In the next year 10 ♂ ♂ and 19 ♀ ♀ from them were bred in mass (B.20, '31). The result was 105 ♀ ♀ and 82 ♂ ♂; the male ratio increased a little over that of the parents' brood. One male among them, orange-red variegated with black, was paired with a normal white female. This mating produced 143 ♂ ♂ and 45 ♀ ♀ ; so the male number amounts to more than thrice that of the female, an enormously high male ratio. The details are listed in table 5.

Table 5

Mating 26, 1932: B'R male crossed with r female.
B'R, B'r, R, r same as in former tables.

SPAWNING INTERVAL	NUMBER OF FRY ABOUT A MONTH OLD	♀ ♀		♂♂		TOTAL		RATIO
						♀ ♀	♂♂	♀ ♂
From July 9 to July 20	98	*B'R* 12 *B'r* 4	*R* 4 *r* 3	*B'R* 16 *B'r* 20	*R* 16 *r* 22	23	74	$\frac{1}{3}$
From July 24 to August 6	72	*B'R* 4 *B'r* 6	*R* 2 *r* 6	*B'R* 10 *B'r* 6	*R* 6 *r* 12	18	34	$\frac{1}{2}$
From August 14 to August 29	210	*B'R* 0 *B'r* 0	*R* 2 *r* 2	*B'R* 9 *B'r* 15	*R* 9 *r* 2	4	35	$\frac{1}{9}$
					Grand total	45♀♀	143♂♂	

The discrimination of sex in our fish according to external aspects which is not possible in young stages may be made only after maturity, when the male manifests its secondary sexual characters, viz., long dorsal fin and wide anal fin with minute horny processes on fin rays (Oka 1931) and when the female lays eggs. From hatching to maturity there is an interval of about one year, so that the young individuals must pass the cold season, many weak ones dying during this hibernation.

Some suspicion might be raised concerning the sex ratios above in that through some unknown cause the majority of the female offspring might die to cause an unusually high male ratio. In the offspring of the third period the rate of mortality is great, thus only 39 adult fishes were reared from 210 fry. But in those of the first period nearly all of the fry became fully grown, and the disturbance of the sex ratio due to death is not observable. The preponderance of the male offspring in this case, therefore, must be due to the special nature of the parents, and this fact alone suffices to deny my former supposition of non-disjunction in the origin of abnormal males. If the latter were the products of non-disjunction and had XXY chromosomes, it should be quite impossible to produce more male offspring than female. In our experiment the male number amounts to more than thrice that of the female. It is clear that our former supposition of non-disjunction must be abandoned, and the hypothesis of sex-reversal must be adopted instead of it.

From all these breeding results we can see that the sex ratio of the offspring of abnormal males crossed with any female of colored varieties varies according to the individuality of the two parents and it differs even in different parents taken from the same litter. Through the continual selection of the abnormal male as parent from the litter where the male ratio is high the male offspring may gradually increase until eventually they are in excess. The increase in the proportion of male offspring generation after generation, that I reported in my last paper (1930) is also probably the result of selection, as I have always chosen the male as parent of the next mating from the litter in which a relatively large number of males was found.

SEX-REVERSED FEMALE OF MALE GENOTYPE

A mass mating of normal strain, 1929, where ten white females (X*r*X*r*) were crossed with five orange-red males (X*R*Y*r*) gave the normal offspring composed of 614 orange-red females, 423 white males and a single exceptional white female. The last one was paired with a single normal orange-red male (X*R*Y*r*), the result of which was quite remarkable, as shown in the next table.

TABLE 5

Exceptional white female crossed with single normal orange-red male (XRYr).
Mating 41, 1930

	ORANGE-RED	WHITE	TOTAL
♀♀	55	0	55
♂♂	55	112	167

If the exceptional female were of normal constitution X*r*X*r* the orange-red and white offspring should be female and male respectively. We see however that half of the orange-red offspring are males: thus among : orange-red individuals one-half, i.e., 55 are male and another half female; while all white individuals, 112 in all, are male. In total the number of the male offspring is thrice that of female.

This singular result may be well explained by supposing that the exceptional female was produced by sex reversal and has X and Y chromosomes. Such a white female X*r*Y*r* mated to a normal orange-red male

XRYr will produce offspring composed of orange-red females XRXr and males XRYr, as well as white males XrYr and YrYr in equal ratio, so that the male number will be thrice that of female if the YY males survive.

If this supposition is correct, half of the white male offspring must have two Y chromosomes and in mating with normal female XX produce male offspring only. To test this supposition, three white males were taken at random and each was mated to three normal females respectively. The results are listed in the next table.

TABLE 6

3 white males from B 41, 1930, crossed each with 3 normal orange-red females.
R, *r* = same as in former tables

NUMBER AND YEAR OF MATING	MALE PARENT	OFFSPRING				TOTAL	
		♀♀		♂♂			
B.17, 1931	A	*R*	14	*R*	8	♀♀	25
		r	11	*r*	15	♂♂	23
B.18, 1931	B	*R*	1	*R*	5	♀♀	6
		r	5	*r*	4	♂♂	9
B 19, 1931	C	*R*	0	*R*	13	♀♀	0
		r	0	*r*	9	♂♂	22

Though since the number of offspring reared from each mating above listed is small the results cannot perhaps be regarded as quite conclusive, yet they are in favor of the supposition just stated. Two males, A and B, produced offspring with a normal sex ratio, while C male produced only males. Ten offspring from the C male, i.e., five orange-reds and five whites were tested by pairing to normal white and orange-red females respectively. The results were quite normal, because the former yielded offspring consisting of orange-red females and white males in criss-cross manner, and the latter both orange-red and white females and males in equal ratio. From these facts it may be inferred that all male offspring of C male are of the normal XY constitution and consequently the C male must have two Y chromosomes.

The orange-red males of the same litter as C male (B.41 '30) were all normal. Three fishes among them were crossed with three normal white females respectively. The results showed normal criss-cross inheritance: all orange-red offspring were females and all whites were males, as shown in table 7.

The single exceptional white female in mating 11, 1931, is the second fish produced by sex reversal which I obtained, and breeding experiments similar to the above stated were repeated with this female: it was paired with a single orange-red male from normal stock. The result was quite similar to that of B.41, '30 (table 8).

TABLE 7

Offspring of 3 orange-red males from B.41, '30, crossed each with 3 normal white females.

NO. AND YEAR OF MATING	ORANGE-RED		WHITE	
	♀♀	♂♂	♀♀	♂♂
B.9, '31	219	0	0	204
B.10, '31	154	0	0	182
B 11, '31	105	0	1	95

TABLE 8

Exceptional white female from B 11, '31, paired with a normal orange-red male. Mating 22, 1932.

	ORANGE-RED	WHITE	TOTAL
♀♀	217	0	217
♂♂	222	374	596

The mode of color inheritance was quite normal, orange-red and white offspring being nearly in equal ratio, as the male parent was heterozygous for its color. One half of the orange-reds were males and the other half females. The total number of males was thrice that of the females as in B.41, '30.

Under the expectation that one half of the white males would yield male offspring only, ten individuals among them were paired each with a single female. The results are shown in table 9.

TABLE 9

Offspring of 10 white males from B.22, '32, crossed with orange-red females.
R = Orange-red. *r* = White.

NO. AND YEAR OF MATING	FEMALE PARENT	NUMBER OF FRY ABOUT A MONTH OLD		ADULT OFFSPRING				TOTAL	
	R	*R*	*r*	*R*♀♀	*R*♂♂	*r*♀♀	*r*♂♂	♀♀	♂♂
B.5, '33	from same litter as male	274	263	32	30	28	31	60	61
B.6, '33	"	173	168	0	119	0	116	0	235
B.7, '33	"	84	102	27	23	35	18	62	41
B.8, '33	"	126	142	0	116	1	116	1	232
B.9, '33	"	202	199	85	68	68	75	153	143
	R								
B.10, '33	from normal stock	254	239	94	104	93	90	187	194
B.11, '33	"	195	219	0	118	0	131	0	249
B.12, '33	"	256	264	101	83	88	93	189	176
B.13, '33	"	306	342	69	76	61	66	130	142
B.14, '33	"	93	86	1	63	0	59	1	122

In four matings B.6, B.8, B.11 and B.14 among ten, only male offspring were produced, and in two matings B.8 and B.14 one single exceptional female was found. The remaining six matings yielded offspring of normal sex ratio, i.e., the female and male nearly in equal proportion. The death rate of young fishes during hibernation is not small, as already stated, and is especially heavy when they are attacked by epidemic disease as in B.5, but in those four matings the greater number of fry was reared up to maturity and there is scarcely any doubt that all females in them were killed and the males alone survived. It is plausible to think that the male parents in those matings carry two Y and no X chromosomes at all.

The fact that four males among ten, i.e., one-half of the whole male offspring carry two Y chromosomes well verifies the supposition that the mother fish of these males, the exceptional white female, is the sex-reversed female of male genotype XY.

For the test of the brother orange-red males (B.22 '32) ten fishes among them were mated to normal white females or orange-red females from the same litter as males. Two matings were unfruitful, the other eight all showed normal inheritance so that all orange-red males of B.22 '32 are to be supposed to have the normal constitution of XY (table 10).

TABLE 10

Orange-red males from B.22, '32 mated to orange-red females of the same litter or to white females from normal stock.

R, *r* same as in former table.

NO. AND YEAR OF MATING	FEMALE PARENT	NUMBER OF FRY ABOUT A MONTH OLD		ADULT OFFSPRING			
	r	*R*	*r*	*R*♀♀	*R*♂♂	*r*♀♀	*r*♂♂
B.18, '33	from normal stock	266	217	247	2	0	197
B.20, '33	"	145	146	138	0	0	135
B.21, '33	"	263	271	187	1	0	179
	R						
B.22, '33	from B.22, '32	297	95	145	67	0	63
B.23, '33	"	442	138	194	100	0	84
B.24, '33	"	454	148	239	114	0	116
B.25, '33	"	216	72	30	22	0	17
B.26, '33	"	252	69	178	63	0	62

Quite similar results were obtained by WINGE (1934) on the sex-reversed female of *Lebistes reticulatus*, which produced in a cross with normal male 21 females and 81 males, i.e., in the ratio of 1:3. One-third of the males were reported to be YY males, and in crosses with normal females produced male offspring only, just as in our case.

It is very interesting to see that in fishes which are well sex-differentiated like Lebistes and Aplocheilus one sex may change to the opposite one, and that YY male may be viable and fertile while it is lethal in Drosophila.

DISCUSSION AND A NEW SUPPOSITION ON SEX DIFFERENTIATION

At present the elucidation of sex differentiation is based on the fundamental conception, proposed by Morgan, of two antagonistic and competing female and male determining factors. Generally it is understood that when the female factor *F* predominates the individual becomes female, while when the male factor *M* prevails it becomes male. But about the locations of these factors in the germ cells the opinions of different authors do not agree.

GOLDSCHMIDT (1934) in his explanation of intersexes in Lymantria (female digamety) locates the *M* factor in X chromosome and the *F* factor in the cytoplasm of the ovum, *FMM* being male and *FM* female. KOSSWIG (1931) to explain sex differentiation in the hybrid between *Platypoecilus maculatus* (female digamety) and *Xiphophorus Helleri*, supposes in the former the *M* factor to be located in autosomes and the *F* in W or Y chromosome; the Z or X chromosome being quite indifferent in sex determination has none of *F* or *M* factor. According to BRIDGES (1925) in Drosophila (male digamety) all autosomes have both female and male genes, and in total the male genes are more effective than the female. The X chromosome in contrast to autosomes contains net female genes, and the sex is differentiated according to the ratio of these two sets of genes. WITSCHI (1929) assumes in European frog (male digamety) that the autosomes have net *M* factor and X chromosome net *F* factor as in Drosophila, and moreover Y chromosome carries a variable *f* factor, the allelomorph of *F* and lower in its strength than the single *M*. WINGE (1934) in the interpretation of sex reversal in Lebistes (male digamety) supposes that the net factor of each autosome is different in quality and strength, some being feminine, and others masculine in various strengths. The X chromosome is feminine, while the Y chromosome is masculine and stronger than a single X. The sex reversal is considered to be caused by the accumulation of autosomes of the same sort in an ovum, whose factors being all feminine or masculine surpass in total the antagonistic effect of sex chromosomes so that an individual XX may be changed to male and the XY to female.

Such diversities of the opinions of different authors about the location of *F* and *M* factors induced me to doubt their real existence. From the facts that in Aplocheilus as well as in Lebistes females of male genotype and males of female genotype may be produced through sex reversal, and that the males of female genotype produce in crosses with normal females offspring with sex ratios varying from all females to a preponderance of males, I am induced to make the following hypothesis on sex differentiation.

The genes which correspond to the primary sexual characters of both sexes are distributed in autosomes, and they become activated by certain definite genes which act as stimulating genes. We may think that the sex differentiation is due to the difference of the quantity of such stimulating genes. When it is greater than a certain limiting value the feminine genes only are activated and the action of masculine genes is suppressed so that the female characters will develop, while on the contrary when it is smaller just the reverse will take place.

The genes concerned in stimulating, which we may call sexual exciters, are located in the sex chromosomes. In the heterogametic male the total sum of sexual exciters in X and Y chromosomes is less than that contained in the two X chromosomes, and in the heterogametic female their total quantity in X and Y or Z and W chromosomes is greater than that in two X or Z chromosomes; in other words, in the former case the X chromosome has greater exciting quantity or potency than the Y and in the latter case we have the reverse. As thus the differentiation of sexes is considered to be caused by the difference of quantity of the same exciting agency there should be a threshold value between the two sex determining quantities and when either of the latter is greater than that value the action of female genes and in the contrary case that of male genes will be stimulated.

Though some external or internal conditions might influence these exciting potencies of sex chromosomes, in normal case their disturbing action is not so great as to cause the total sum of the potencies of sex chromosomes to pass over the threshold value and produce abnormal sex differentiation.

According to my opinion the sex reversal in our fish may be explained by an unusual disturbance of the potency of the X chromosome. In our breeds of colored varieties, I think, the X chromosome loses the constancy of its potency by some unknown cause, and so fluctuates always in varying range that in some fishes which happened to have lower fluctuants of the X chromosome the total sum of their potencies falls under the threshold value, so that the masculine genes only are activated notwithstanding their female genotype, and the sex is reversed.

The range of fluctuation is supposed not to be equal in all X chromosomes and it varies even in those of offspring produced from the same parents. The variation of the number of sex-reversed males due to the individuality of both parents in the offspring of the XX male crossed with the female of colored varieties will be the result of the varying ranges of fluctuation in the X chromosomes of the latter. The wider the range of their fluctuation in both parents, the greater number of males will be produced.

From the fact that in crosses with wild females any sex-reversed male yields generally female offspring only, rarely together with a few males, we may infer that the range of fluctuation is not very wide in negative direction, hence most of the lower fluctuants, excepting the lowest ones, in combination with a normal X chromosome of the wild species are unable to depress the total sum of potencies so much as to let it pass over the threshold value and effect sex reversal.

The fluctuation of the X chromosome in our breed, in my view, would have taken place at first in one of two X chromosomes, and on account of many years' close inbreeding all colored varieties in our breeds came to get two fluctuating X chromosomes, whereupon some females which happened to carry two lower X chromosomes were first detected as the sex-reversed ones. By constant breeding of the sex-reversed males selected out from the litters where their number is high, the fluctuation of X chromosome may be shifted further in the negative direction, so that the number of the male offspring will be increased. The matings in table 5 are the results of such processes, where the excess of males may be found.

Probably the fluctuation of the X chromosome might be influenced easily by outer conditions, and the difference of the rate of male production from the same parents in different seasons is to be explained as the effect of different climate.

The fluctuation takes place in both directions either positive or negative, starting from normal value. The effect of the positive fluctuation cannot be recognized in the offspring of sex-reversed males crossed with normal females as then both parents carry X chromosomes only, and the increase in the potency of that chromosome results in nothing but to produce the females which are not different at all from the normal ones.

The sex-reversed female of male genotype XY is to be explained as the effect of the increase in the total potency of X and Y chromosomes. When the total sum of potencies increases and passes over the threshold value to the female side, a female will be produced in spite of the male genotype. Whether this increase of potency is caused by the fluctuation occurring in the X chromosome only or in both X and Y is not yet fully decided. But even through the consideration of the fluctuation in the X chromosome only the fact is easily understood. The single X chromosome of extreme fluctuation on the positive side, which will very rarely occur, accompanied by a Y chromosome, may have sufficient total potency to excite the female genes only. The rarity of sex-reversed females favors this supposition.

The experimental results of the sex reversal phenomenon in our fish are thus fairly explainable by the supposition that sex differentiation is due to differences in the total quantities of exciting factors in the sex chromosomes, and the fluctuation of the potency of the X chromosome.

Now to test how far this supposition of sex differentiation is conformable to the other facts about sex differentiation, I will try in the following to interpret some well known complex facts based on this supposition.

The most perplexing facts are those of Goldschmidt's intersexes and sex reversal in the hybrids between the different local races of *Lymantria dispar*. The results of the crosses designated as "Basic" by him and the crosses between the very weak and strong races (GOLDSCHMIDT 1934) can easily be interpreted on the basis of our hypothesis with an auxiliary one that the maternal cytoplasm influences slightly the potencies of sex chromosomes: the cytoplasm of the strong race strengthens and that of the weak weakens the potencies of sex chromosomes.

To simplify the explanation we may assume schematically some numbers for the potencies of sex chromosomes and the threshold value in different races:

Japanese or strong race.............	X20 Y40 = 60, X20 X20 = 40, the threshold value = 52.
European or weak race	X25 Y37 = 62, X25 X25 = 50, the threshold value = 55.
Very weak race......	X26 Y30 = 56, X26 X26 = 52, the threshold value = 53.

In the hybrids between these races of different potencies of X and Y chromosomes there will appear some individuals in which the total potency of sex chromosomes is enough large to excite one group of genes, either female or male, in the autosomes introduced from one parent, so that the corresponding sexual characters will develop. For the sexual genes in another group of autosomes introduced from another parent it is at the threshold value or very near to it, so that some characters of both sexes may develop, and in such a case a female or male intersex will be produced. Sex reversal will be produced when the total potency of sex chromosomes passes over the threshold values in both parents to that side, male or female, which is opposite to the sexual constitution of the hybrid.

In the next table the statements of GOLDSCHMIDT on the different crosses are cited in the first column and the respective interpretation for each cross is described in the second. The cytoplasmic influence is denoted by the number 2; when the female parent is from the strong race 2 is added, and when it is from the weak or very weak race 2 is subtracted from the total sum of potencies in each zygote.

TABLE 11

Interpretations of Goldschmidt's basic crosses and crosses between strong and very weak races of Lymantria dispar.

J = JAPANESE STRONG RACE E = EUROPEAN WEAK RACE	j = AUTOSOME SET FROM JAPANESE RACE e = AUTOSOME SET FROM EUROPEAN RACE ⚥ = INTERSEX. Rev = SEX REVERSAL
1. J♀ × E♂ = F_1 normal	X20 Y40 × X25 X25 = X20 X25, X25 Y40 45+2 = 47 65+2 = 67 ♂ ♀
2. E♀ × J♂ = F_1♂ normal, ♀ intersex	X25 Y37 × X20 X20 = X25 X20, X20 Y37 45−2 = 43 57−2 = 55 ♂ j♀, e⚥
3. $(J \times E)^2$ = ♀ normal, ♂ up to ½ of their number intersexual	X25 Y40 × X20 X25 = X20 Y40, X25 Y40, X20 X25, X25 X25 60+2 = 62 65+2 = 67 45+2 = 47 50+2 = 52 ♀ ♀ ♂ e♂, i⚥

J=JAPANESE STRONG RACE E=EUROPEAN WEAK RACE	j=AUTOSOME SET FROM JAPANESE RACE e=AUTOSOME SET FROM EUROPEAN RACE ⚥=INTERSEX. Rev=SEX REVERSAL
3a. (J×E)×(E×J)=exactly like No. 3	X25 Y40×X25 X20= X20 Y40, X25 Y40 X20 X25 X25 X25 60+2=62 65+2=67 45+2=47 50+2=52 ♀ ♀ ♂ e♂, j⚥
4. (E×J)^2=♂ normal, ♀ ½ normal and ½ intersexuals	X20 Y37×X20 X25= X20 X20, X20, X25, X25 Y37, X20 Y37 40−2=38 45−2=43 62−2=60 57−2=55 ♂ ♂ ♀ j♀, e⚥
4a. (E×J)×(J×E)=exactly like No. 4	X20 Y37×X25 X20=same as No. 4
5. J×(J×E), J×(E×J): always normal	X20 Y40×X20 X25= X20 X20, X20 X25, X20 Y40, X25 Y40 40+2=42 45+2=47 60+2=62 65+2=67 ♂ ♂ ♀ ♀
6. E×(J×E),E×(E×J): exactly like F$_2$ No. 4, namely males normal, females ½ normal, ½ intersexual	X25 Y37×X20 X25= X20 X25, X25 X25, X25 Y37, X20 Y37 45−2=43 50−2=48 62−2=60 57−2=55 ♂ ♂ ♀ j♀, e⚥
7a. (J×E)×J=all normal	X25 Y40×X20 X20=X25 X20, X20 Y40 45+2=47 60+2=62 ♂ ♀
7b. (J×E)×E= ♀ normal, ♂ all intersexual	X25 Y40×X25 X25=X25 Y40, X25 X25 65+2=67 50+2=52 ♀ e♂, j⚥
8a. (E×J)×J=all ♂ normal, ♀ intersexual	X20 Y37×X20 X20=X20 X20, X20 Y37 40−2=38 57−2=55 ♂ j♀, e⚥
8b. (E×J)×E=all normal	X20 Y37×X25 X25=X20 X25, X25 Y37 45−2=43 62−2=60 ♂ ♀
a. Very weak ♀×Strong ♂=only ♂	X26 Y30×X20 X20=X20 X26, X20 Y30 46−2=44 50−2=48 ♂ Rev♂
b. Strong ♀×Very weak ♂=normal	X20 Y40×X26 X26=X20 X26, X26 Y40 46+2=48 66+2=68 ♂ ♀
c. Very weak ♀×F$_1$ ♂ from b=3♂:1♀	X26 Y30, X20 X26= X20 X26, X20 Y30, X26 X26, X26 Y30 46−2=44 50−2=48 52−2=50 56−2=54 ♂ Rev ♂ ♂ ♀
d. (Strong ♀×Very weak ♂)^2=2♀, 1♂, 1♀ by sex-rev. (hardly viable)	X26 Y40×X20 X26= X26 X20, X20 Y40, X26 X26, X26 Y40 46+2=48 60+2=62 52+2=54 66+2=68 ♂ ♀ Rev ♀ ♀
e. (Strong ♀×Very weak ♂) ♀×Very weak ♂=only ♀	X26 Y40×X26 X26=X26 X26, X26 Y40 52+2=54 66+2=68 Rev ♀ ♀

The appearance of abnormal sex in *D. melanogaster* (BRIDGES 1922, 1925) may also be elucidated on the basis of our present supposition. The triploid intersex carries triploid autosomes and two X chromosomes (3A, 2X). The total of the potencies of two X chromosomes may be here considered to be equal or very near to the threshold value for three sets of autosomes, and consequently both sexual characters are revealed. The super-female (2A, 3X) and male (3A, X) are considered to be caused by extremely excessive and scanty quantities of the exciting factor respectively, of which the sterility is the result.

In bees and many other Hymenoptera the diploid egg develops into a female and the haploid into a male. These facts are intelligible when we assume a cytoplasmic influence upon the potencies of sex chromosomes. We suppose that the cytoplasm in Hymenoptera resists the exciting action of sex chromosomes and weakens it somewhat. In a diploid egg the sum of the potencies of two sex chromosomes, 2x, decreased by the quantity c, the cytoplasmic resistance, i.e. 2x-c, might still be greater than the threshold value for two sets of autosomes, and stimulate the female genes to their activity, while in the haploid form x-c is less than the threshold value for one set of autosomes, and the male genes only will be excited.

Thus the hypothesis of quantitative differences in the degree of sensitivity of the male and female sexual genes in autosomes and corresponding differences in the potencies of sex chromosomes in different sexes explains well many complex facts of sex differentiation, and I think that it is the general mode of sex differentiation.

Through this hypothesis the phylogenetic relationship among the hermaphrodite, rudimentary hermaphrodite (KOSSWIG 1931, WITSCHI 1929) and the gonochorist may be well cleared up. In the hermaphrodite the sensitivity of male and female genes to stimulating action is equal, and all of them are consequently excited to the same degree. In the rudimentary hermaphrodite, however, the sensitivity of sexual genes and the corresponding total potency of sex chromosomes is different in male and female sexes but this difference is small and the total potency of sex chromosomes in each sex draws very near to the threshold value. In such animals some slight influence of outer or inner conditions will induce the disturbance of sex differentiation, sex reversal or intersexuality being easily produced. In the gonochorist this difference is so great that normally any condition is unable to cause such sexual disturbances.

The three sexual types i.e., Protenor ♀XX♂XO, Drosophila ♀XX-♂XY and Abraxas type ♀XY♂XX show according to our hypothesis only the differences in respect to the accumulation of stimulating genes in two sex chromosomes. In the Protenor type all genes are contained in the X chromosome only and in the latter two types they are distributed between two chromosomes X and Y. In the Drosophila type the X chromosome carries a greater number of genes than Y, and in the Abraxas type on the contrary the Y chromosome is greater in its potency than the X. Crossing over of the stimulating genes, if possible, might produce one type from another. The difficulty that opposite types of sex determination appear in very nearly related species, for example, the Drosophila type in Lebistes and the Abraxas type in Platypoecilus may thus be easily explained.

SUMMARY

Further breeding investigations on the abnormal males of *Aplocheilus latipes*, which were assumed in my former paper (1927) to be the products of non-disjunction of the sex chromosomes are described. These males produce in crosses with normal females offspring in which the number of females always exceeds that of males in varying ratios.

The sex ratios in these offspring vary according to the individualities of male and female parents. The different males from the same litter yield offspring of different sex ratios, and also the different females mated to the same male produce offspring of varying sex ratio.

Temperature influences the sex ratio which may differ in the offspring of the same parents produced early in the year or during mid-summer. Generally under a hotter climate the number of male offspring is greater than under a colder one.

By continued breeding of males selected out from the litters with high ratios of males the number of males increases until eventually some males are obtained whose male offspring far exceed the females in number. This fact denies the supposition of non-disjunction as accounting for the origin of abnormal males, so that it must be discarded and the alternative of sex reversal adopted.

The sex-reversed female of male genotype XY was found. It produced in crosses with normal males female and male offspring in the ratio of 1:3. One-third of its male offspring were males of the constitution YY, and these produced only male offspring.

For the interpretation of these results a new hypothesis of sex differentiation is proposed. The female and male primary sexual characters have all their own respective corresponding genes; these are distributed in the autosomes, and are set into activity by a certain amount of stimulating genes. The degree of sensitivity of female and male genes to the stimulating genes is various, and the female genes require a greater amount of stimulation than the male genes to become active. Sex differentiation is caused by differences in quantity of the stimulating genes. When the difference is great the female genes alone are activated and the action of male genes suppressed, while if it is small just the reverse takes place. Between these

two quantities is a threshold value, above or below which the female or male genes are stimulated to produce female or male respectively. The genes of stimulation in the gonochorist are located in the sex chromosomes, and the total stimulating capacity or potency is greater in female sex chromosomes than in male ones.

Sex reversal in our fish and the differences in the sex ratios among the offspring of sex-reversed males are explained by the fluctuation of the stimulating power or potency of the X chromosome.

Other abnormal facts of sex differentiation are explained on the basis of our present hypothesis.

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THE THEORY OF MULTIPLE-STRAND CROSSING OVER[1]

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THE PROBLEM

According to the theory of the chiasmatype as originally formulated by Janssens (1909), interchange between homologous chromosomes takes place when each is already split longitudinally, but at any level only two of the four strands exchange parts. It was pointed out by Muller (1916) and Bridges (1916) that the theory could be demonstrated genetically if it could be shown that in eggs which have retained two maternal strands, one strand may be a crossover and the other a non-crossover, or both crossovers but not at the same level. Such individuals were obtained by Muller and Bridges; and although in these cases there was, as Muller pointed out, the possibility that the extra strands had arisen by non-disjunction prior to maturation, the correctness of Janssens's theory has since been demonstrated by the regular occurrence of such individuals in races with attached X's or high non-disjunction and in triploids (Anderson 1925b, Bridges and Anderson 1925, L. V. Morgan 1925, Redfield 1930, Sturtevant 1931).

If crossing over occurred at a two-strand stage, each chromatid would be identical with one of the other chromatids of the tetrad and the complement of the remaining two; hence the enumeration of the strands and the determination of how they are combined in the tetrad would be a simple matter. In four-strand crossing over, the strands recovered are presumably still a random sample of all the strands; but there are two complications. (1) Each of the missing strands is not necessarily either identical with or the complement of the strand recovered. (2) There is the possibility that crossing over may have occurred between sister strands; and this would not be directly detectable.

A complete theory of crossing over must take into account the missing strands in each tetrad and the unrecognizable crossings over. At first

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sight this may seem like a search for the substance of things hoped for, the evidence of things not seen; nevertheless the nature of the strands and tetrads can be deduced by calculation from the experimental data.

MATHEMATICAL METHOD

Definitions

Rank. The number of levels at which crossing over occurs in a strand or tetrad will be termed its rank. Non-crossovers are of rank 0, singles of rank 1, and so forth.

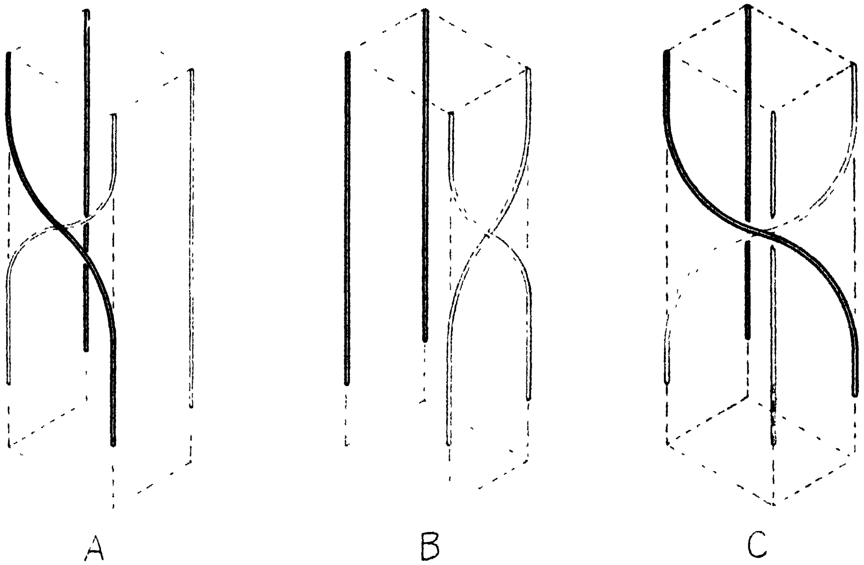


FIGURE 1. Types of single crossing over. A, lateral, involving homologous strands; B, lateral, involving sister strands; C, diagonal, involving homologous strands.

Types of single crossing over. A distinction must be made between homologous and sister-strand crossing over; also between lateral and diagonal crossing over if the strands of a tetrad are arranged along the edges of a quadrilateral prism (figure 1).

Types of multiple crossing over. In tetrads of rank 2, crossing over will be termed regressive, progressive, or digressive according to whether both or one or neither of the strands that cross over at the first level is involved in the crossing over at the second level (figure 2). A tetrad of higher rank than 2 may be mixed in type. In figure 3 are shown the various types of tetrads of ranks 2 and 3, but no distinction is made between lateral and diagonal crossing over.

A tetrad can give rise to strands of its own rank or of lower ranks but not to strands of higher rank. The rank of an emerging strand may however be greater than the number of levels at which any one of the original strands that enter the tetrad crosses over (figure 3, tetrad G6).

Association of chromatids. Completely random association of chromatids in crossing over implies (1) that at any given level any two chromatids of a tetrad are equally likely to cross over (this may be termed random local association, or random occurrence of crossing over); (2) that the two chromatids which cross over at one level do not determine which shall cross over at other levels (random recurrence of crossing over).

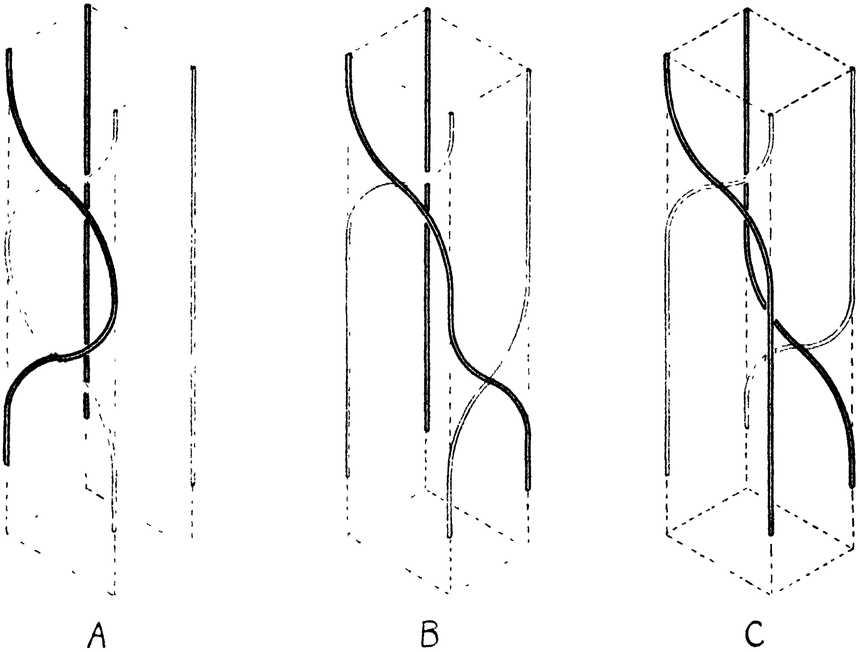


FIGURE 2. Types of double crossing over A, regressive, B, progressive; C, digressive

We shall first work out the theory of crossing over for completely random association; and then generalize it for cases where either occurrence, or recurrence, or both are not random.

Case 1. Recurrence random, chance of detecting crossing over constant.

A. Random occurrence (free sister-strand crossing over)

A tetrad of rank 0 can give rise only to non-crossover strands. In a tetrad of rank 1, half the strands will be crossovers and half non-crossovers, so that the chance of obtaining a strand of rank 1 will be 1/2, if elimination into polar bodies is a random matter. But not all the crossover strands will be recognizable as such: those resulting from crossing over between sister strands will remain unaltered. Since on a random basis one-third of the exchanges at a given level will be between sister

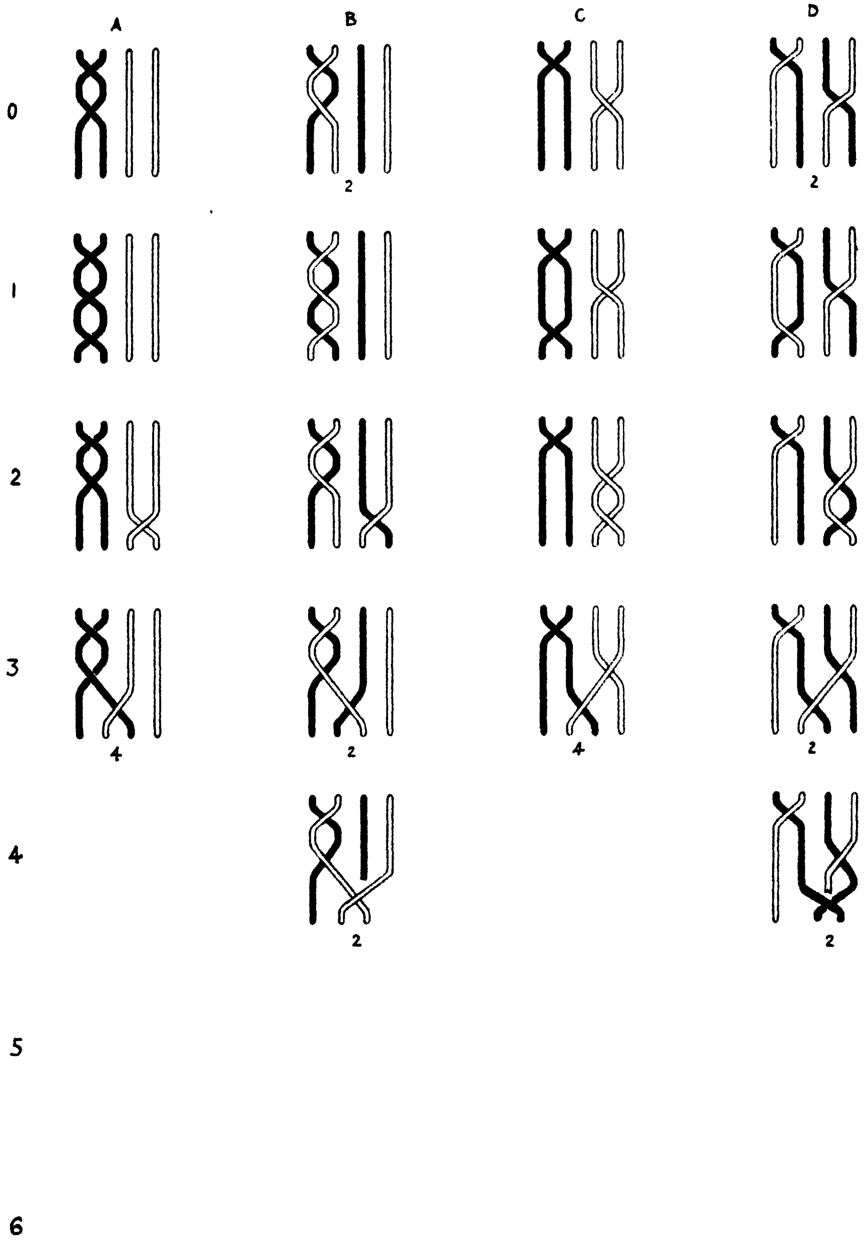
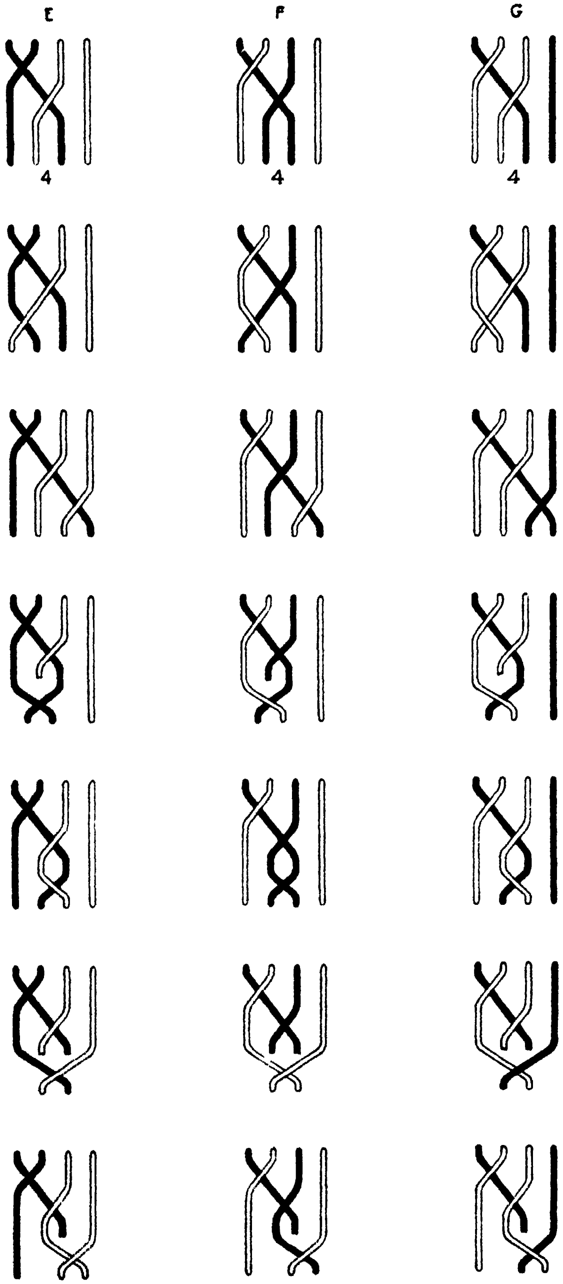


FIGURE 3. Types of double and triple crossing over. In the uppermost horizontal row (marked 0) are shown the types of tetrads of rank 2; the figure beneath each tetrad indicates its relative frequency. Beneath each tetrad of rank 2 are shown (rows 1–6) the tetrads that result when crossing over occurs in a third region. The numeral under each tetrad of rank 3 indicates its relative frequency among tetrads of rank 3 in the same vertical column; its frequency among all tetrads of rank 3 is the product of this number by the frequency of the tetrad of rank 2 from which it is derived. Where no number is indicated, 1 is understood. All frequencies in this figure are based on the assumption of random occurrence and recurrence.


E
F
G
4
4
4

strands, only the other two-thirds will be recognizable. Hence the chance that a tetrad of rank 1 will give rise to a chromatid recognizable as a single crossover will be $1/2 \cdot 2/3 = 1/3$; that is, the observed frequency of crossing over in any region short enough to have only one crossing over at a time will be one-third of the true frequency.

If recurrence is random, the chances of detecting crossing over at different levels are independent. Hence the chance that a tetrad of rank 2 will result in an individual recognizable as a double crossover will be $1/3 \cdot 1/3 = 1/9$; the chance that a tetrad of rank 3 will result in an individual recognizable as a triple crossover will be $(1/3)^3 = 1/27$; and in general the chance that a tetrad of rank r will result in an individual recognizable as an r-ple crossover will be $(1/3)^r$.

The chance that a tetrad of rank 2 will result in an individual which is a recognizable crossover only in the first region is $1/3 \cdot 2/3 = 2/9$; and this is also the chance that the tetrad will give rise to an individual which is a recognizable crossover in the second region only. The chance that an individual will emerge which is a non-crossover or an apparent non-crossover will be $2/3 \cdot 2/3 = 4/9$.

Of strands derived from tetrads of rank 3, those that are recognizable as triple crossovers will be $(1/3)^3 = 1/27$; those that are recognizable as crossovers in the first two regions only will be $(1/3)^2 \; 2/3 = 2/27$, and this will also be the frequency of strands that are recognizable crossovers in regions 1 and 3 only, or in 2 and 3 only. The frequency of recognizable crossovers in region 1 only will be $1/3 \; (2/3)^2$; and this will also be the frequency of recognizable crossovers in region 2 only, or in region 3 only. Finally, the frequency of non-crossovers and apparent non-crossovers together will be $(2/3)^3 = 8/27$.

In general, if a tetrad is of rank r, the chance that it will give rise to a chromatid which is a recognizable crossover in some specified k of the r regions will be $(1/3)^k \; (2/3)^{r-k}$.

By this method we can deduce from a set of crossover data the frequencies of tetrads of different classes. In table 1, the experimental data in the first line are taken from a cross of BRIDGES (cited by WEINSTEIN 1918). Since each individual must have been derived from a tetrad of at least its own rank, the triples must be derived from tetrads of rank 3; for there were probably no tetrads of higher rank, because each of the three regions is too short to allow more than single crossing over within itself.

The one triple in the experimental data must represent 27 tetrads of rank 3. These tetrads must have produced in addition to the 1 triple, 2 individuals in each of the three rank-2 classes, 4 individuals in each class of rank 1, and 8 non-crossovers. If we subtract each of these from the total in the corresponding class, the remainder represents those individuals in that class derived from tetrads of rank lower than 3.

We now turn to the 2, 3 class. The remainder in this class must be derived from tetrads that were crossovers in regions 2 and 3 only. From these tetrads emerged 41 individuals of class 2, 3; 82 that were crossovers in region 3 only, 82 that were crossovers in region 2 only, and 164 non-crossovers. In a similar way, we can calculate the individuals derived from the 1, 3 and the 1, 2 tetrads; and if we subtract all the individuals derived from tetrads of rank 2, the remainders represent the individuals derived from tetrads of lower ranks. The individuals in each crossover class of rank 1 constitute only 1/3 of those derived from the tetrads of the same class; the other 2/3 must have been non-crossovers and must be subtracted from the observed non-crossovers.

TABLE 1

Frequencies of tetrads calculated for random association including sister-strand crossing over

REGIONS OF CROSSING OVER	0	1	2	3	12	13	23	123
Observed frequencies	9927	1949	1664	1651	88	207	43	1
Frequencies and distribution of tetrads of rank 3	8	4	4	4	2	2	2	*1*
Remainders	9919	1945	1660	1647	86	205	41	
Frequencies and distribution of tetrads	164		82	82			*41*	
of rank 2	820	410		410		*205*		
	344	172	172		*86*			
Remainders	8591	1363	1406	1155				
Frequencies and distribution of tetrads	2310			*1155*				
of rank 1	2812		*1406*					
	2726	*1303*						
Remainder	*743*							
Corrected frequencies of tetrads	743	4089	4218	3465	774	1845	369	27

Thus we arrive at the italicized figures along the diagonal, which give the number of individuals of each class derived from tetrads of the same class.

These frequencies however are not the frequencies of the tetrads of each class; for, as we have seen, in a tetrad of rank r, only $(1/3)^r$ of the emerging strands are of the same rank, r. Hence it is necessary to multiply the remainder in each single crossover class by 3, in each double crossover class by 9, in each triple crossover class by 27. The results, in the lowest horizontal line, give the true number of tetrads in each class on the assumption of random occurrence and recurrence with sister-strand crossing over.

B. No sister-strand crossing over

If we assume that there is no crossing over between sister strands but that otherwise association of strands is random, crossing over may occur in one of four ways at any level (ab, ab′, a′b, a′b′, where a is the sister strand of a′ and b of b′). The chance that the strand recovered is a crossover is 1/2, and this is also the chance of detecting the crossing over at that level in the tetrad, since every crossing over is recognizable once it is obtained.

Of the strands derived from a tetrad of rank r, those that are also of rank r will be $(1/2)^r$, those that are crossovers in any $(r-1)$ specified regions will be $(1/2)^{r-1} \cdot 1/2$, and so forth. In general the chance that a tetrad of rank r will give rise to a strand which is a crossover in some specified k of the r regions will be $(1/2)^k\ (1/2)^{r-k} = (1/2)^r$. That is, all the classes derived from tetrads of a given kind occur with equal frequency; since the frequency is independent of k, the number of regions in which the strands are crossovers.

This procedure is illustrated in table 2.

TABLE 2

Frequencies of tetrads calculated for random association without sister-strand crossing over.

REGIONS OF CROSSING OVER	0	1	2	3	12	13	23	123
Observed frequencies	9927	1949	1664	1651	88	207	43	1
Frequencies and distribution of tetrads of rank 3	1	1	1	1	1	1	1	*1*
Remainders	9926	1948	1663	1650	87	206	42	
Frequencies and distribution of tetrads of rank 2	42		42	42			*42*	
	206	206		206		*206*		
	87	87	87		*87*			
Remainders	9591	1655	1534	1402				
Frequencies and distribution of tetrads of rank 1	1402			*1402*				
	1534		*1534*					
	1655	*1655*						
Remainder	*5000*							
Corrected frequencies of tetrads	5000	3310	3068	2804	348	824	168	8

The remainder in each class will now have to be multiplied by 2^r, where r is the rank of the class, to give the number of tetrads of that class.

C. The general case

It is conceivable that sister chromatids cross over with a frequency which differs from what it would be on a random basis but is not 0. The chance of detecting a crossing over in a region would then be neither 1/3 nor 1/2; let it be designated by p. Then if p has the same value in every region of the chromosome, the frequencies of tetrads can still be calculated by the method explained above.

A formula for this procedure can be derived as follows.

Let the chromosome be divided into regions so short that no crossing over of rank higher than 1 occurs within each. Let $a_0, a_1, a_2, \cdots a_n$ be the observed frequencies of individuals that are crossovers in 0, 1, 2, · · · n regions. It should be noted that each subscript represents the cardinal number of crossings over, not the ordinal number of the region in which crossing over takes place. Let $x_0, x_1, x_2, \cdots x_n$ represent the number of tetrads that are crossovers in 0, 1, 2, · · · n regions.

The chance that a tetrad of rank r will give rise to a strand which is a crossover in some specified k of these r regions is $p^k\ (1-p)^{r-k}$, or $p^k\ q^{r-k}$ where $q = 1-p$. The number of ways in which these k regions can be specified is $[r(r-1)(r-2) \cdots (r-k+1)]/(1 \cdot 2 \cdot 3 \cdot \cdots k)$. Hence the total chance of obtaining a crossover strand of rank k from a tetrad of rank r is the product of these two expressions.

We can now form the following equations.

$$
\begin{array}{lllllllll}
a_0 = x_0 + qx_1 + & q^2x_2 + & q^3x_3 + & q^4x_4 + & q^5x_5 + & \cdots & + & q^nx_n \\
a_1 = & px_1 + 2pqx_2 + & 3pq^2x_3 + & 4pq^3x_4 + & 5pq^4x_5 + & \cdots & + & npq^{n-1}x_n \\
a_2 = & & p^2x_2 + 3p^2qx_3 + & 6p^2q^2x_4 + & 10p^2q^3x_5 + & \cdots & + & C_2^np^2q^{n-2}x_n \\
a_3 = & & & p^3x_3 + 4p^3qx_4 + & 10p^3q^2x_5 + & \cdots & + & C_3^np^3q^{n-3}x_n \\
a_4 = & & & & p^4x_4 + 5p^4qx_5 + & \cdots & + & C_4^np^4q^{n-4}x_n \\
a_5 = & & & & p^5x_5 + & \cdots & + & C_5^np^5q^{n-5}x_n \\
\cdots & & & & & & & \cdots \\
a_n = & & & & & & & p^nx_n
\end{array}
$$

In these $(n+1)$ equations there are $(n+1)$ unknowns, the x's; for the a's are observed frequencies and p is determined by the assumptions as to the chance of detecting a crossing over within a region. Hence if the equations are solved simultaneously, the x's can be evaluated.

But the value of x_0 can be obtained without solving for the other x's. For if the equations are multiplied respectively by 1, $-q/p$, q^2/p^2, $-q^3/p^3$, $\cdots$ $(-q/p)^n$, and then added together, the coefficients of every x except x_0 will add up to 0. Hence

$$x_0 = a_0 - \frac{q}{p}a_1 + \left(\frac{q}{p}\right)^2 a_2 - \left(\frac{q}{p}\right)^3 a_3 + \cdots + \left(-\frac{q}{p}\right)^n a_n. \qquad (1)$$

In this procedure a_0 has been used to represent the frequency of the non-crossovers. But it may be used for the frequency of any class; for example, the crossovers in region 1, or the crossovers in regions 1 and 3, provided that the classes which are not crossovers in these regions are excluded. Then in the data so selected, $a_1, a_2, a_3, \cdots a_n$ represent the frequencies of the classes that are crossovers in the same region or regions as the a_0 class and in 1, 2, 3, $\cdots$ n additional regions; and $x_0, x_1, x_2, x_3, \cdots x_n$ are the frequencies of the tetrads which are crossovers in the same regions as the a_0 class and in 0, 1, 2, 3, $\cdots$ n additional regions.

The solution for x_0 will now give the number of tetrads of any specified class that gave rise to strands of the same class. But (unless this class is the non-crossovers) x_0 is not the total number of tetrads of the class; for there must have been others that gave rise to strands of lower rank and these have been excluded from the calculations. Since the proportion of tetrads of rank r that give rise to strands of the same rank is p^r, it is necessary to multiply x_0 by $1/p^r$ to get the number of all the tetrads of the class in question. This frequency is therefore

$$X=\frac{1}{p^r}x_0=\frac{1}{p^r}\left[a_0-\frac{q}{p}a_1+\left(\frac{q}{p}\right)^2a_2-\left(\frac{q}{p}\right)^3a_3+\cdots+\left(-\frac{q}{p}\right)^na_n\right]$$

$$=\frac{1}{p^r}\left[a_0-\left(\frac{1-p}{p}\right)a_1+\left(\frac{1-p}{p}\right)^2a_2\right.$$

$$\left.-\left(\frac{1-p}{p}\right)^3a_3+\cdots+\left(-\frac{1-p}{p}\right)^na_n\right]. \qquad (2)$$

where a_0 is the observed frequency of the class in question, and $a_1, a_2, a_3, \cdots a_n$ the observed frequencies of classes of additional rank 1, 2, 3, $\cdots$ n (WEINSTEIN 1928, 1932a).

For random occurrence and recurrence with sister strand crossing over, $p=1/3$ and the formula becomes

$$X=3^rx_0=3^r[a_0-2a_1+4a_2-8a_3+\cdots+(-2)^na_n]. \qquad (2a)$$

If sister-strand crossing over is entirely excluded but occurrence and recurrence are otherwise random $p=1/2$ and the formula becomes

$$X=2^rx_0=2^r[a_0-a_1+a_2-a_3+\cdots+(-1)^na_n]. \qquad (2b)$$

Case 2. Recurrence random, chance of detecting crossing over variable.

In case 1, it was assumed that p, the chance of detecting crossing over, is the same for all regions. It is theoretically possible however that the chance is not invariant, for sister chromatids might cross over in some regions and not in others, or more frequently in some regions than in others. Such differences might be caused by local conditions like proximity to the end of the chromosome or to the spindle fibre or to inert regions or perhaps to particular genes.

In such cases we may designate the chance of detecting crossing over in regions 1, 2, 3, $\cdots$ n as p_1, p_2, p_3, $\cdots$ p_n. Let $q_1=1-p_1$, $q_2=1-p_2$, $q_3=1-p_3$, $\cdots$ $q_n=1-p_n$.

If recurrence is random, the chance that a tetrad of rank r will give rise to a strand which is a crossover in some specified k of the r regions is the product of the p's for the k regions and the q's for the remaining regions. Thus the chance that a tetrad which is a crossover in regions 1, 2, and 3 will give rise to a strand which is a crossover only in regions 1 and 3 is $p_1\,p_3\,q_2$.

The frequencies of tetrads can now be calculated as in table 3.

TABLE 3

Frequencies of tetrads calculated for random association except that sister strands cross over only in region 1

REGIONS OF CROSSING OVER	0	1	2	3	12	13	23	123
Observed frequencies	9927	1949	1664	1651	88	207	43	1
Frequencies and distribution of tetrads of rank 3	2	1	2	2	1	1	2	*1*
Remainders	9925	1948	1662	1649	87	206	41	
Frequencies and distribution of tetrads of rank 2	41		41	41			*41*	
	412	206		412		*206*		
	174	87	174		*87*			
Remainders	9298	1655	1447	1196				
Frequencies and distribution of tetrads of rank 1	1196			*1196*				
	1447		*1447*					
	3310	*1655*						
Remainder	*3345*							
Corrected frequencies of tetrads	3345	4965	2894	2392	522	1236	164	12

A formula for this procedure can also be deduced.

Let the observed frequency of the non-crossovers be represented by b_0; of the singles by b_1, b_2, b_3, $\cdots$ b_n; of the doubles by b_{12}, b_{13}, $\cdots$ b_{23}, b_{24}, $\cdots$ b_{34}, $\cdots$ $b_{(n-1)n}$; and similarly for the remaining classes. Each subscript now represents not, as before, the cardinal number of crossings over, but the ordinal numbers of the regions in which crossing over occurs. The frequencies of tetrads of various classes can be denoted by y with corresponding subscripts; thus y_0 is the frequency of non-crossover tetrads, y_1 of tetrads that are crossovers in region 1 only, and so forth.

We can now form a set of equations analogous to those in case 1. For the sake of simplicity the equations will be given for only three regions; but the method is applicable to any number of regions.

$$\begin{aligned}
b_0 &= y_0+q_1y_1+q_2y_2+q_3y_3+q_1q_2y_{12}+q_1q_3y_{13}+q_2q_3y_{23}+q_1q_2q_3y_{123}\\
b_1 &= p_1y_1+p_1q_2y_{12}+p_1q_3y_{13}+p_1q_2q_3y_{123}\\
b_2 &= p_2y_2+p_2q_1y_{12}+p_2q_3y_{23}+p_2q_1q_3y_{123}\\
b_3 &= p_3y_3+p_3q_1y_{13}+p_3q_2y_{23}+p_3q_1q_2y_{123}\\
b_{12} &= p_1p_2y_{12}+p_1p_2q_3y_{123}\\
b_{13} &= p_1p_3y_{13}+p_1p_3q_2y_{123}\\
b_{23} &= p_2p_3y_{23}+p_2p_3q_1y_{123}\\
b_{123} &= p_1p_2p_3y_{123}
\end{aligned}$$

These equations can now be solved for the y's. But again it is possible to obtain the value of y_0 without solving for the other y's; for if the equations are multiplied respectively by

$$1,\ -\frac{q_1}{p_1},\ -\frac{q_2}{p_2},\ -\frac{q_3}{p_3},\ \frac{q_1q_2}{p_1p_2},\ \frac{q_1q_3}{p_1p_3},\ \frac{q_2q_3}{p_2p_3},\ -\frac{q_1q_2q_3}{p_1p_2p_3},$$

and then added together, the coefficients of the y's in every column, except y_0 will add up to 0. Hence

$$y_0=b_0-\left(\frac{q_1}{p_1}b_1+\frac{q_2}{p_2}b_2+\frac{q_3}{p_3}b_3\right)+\left(\frac{q_1q_2}{p_1p_2}b_{12}+\frac{q_1q_3}{p_1p_3}b_{13}+\frac{q_2q_3}{p_2p_3}b_{23}\right)-\frac{q_1q_2q_3}{p_1p_2p_3}b_{123}. \tag{3}$$

If the b_0 class is of rank 0, then y_0 is the number of non-crossover tetrads. But as in case 1, the method can be applied to part of the data: then b_0 is the frequency of a crossover class and y_0 is the number of tetrads of this class that gave rise to chromatids of the same class. Hence y_0 must be divided by the product of the p's for the regions in which the class is a crossover. These regions are not any of those numbered from 1 to 3, for the b_0 or y_0 class is not a crossover in 1 or 2 or 3. Hence the regions in which the class is a crossover can be numbered separately from 1 to r, where r is the rank of the class. The chance of detecting crossing over in each of these r regions may be denoted by p' with the proper subscript; and the total number of tetrads of the class now becomes

$$X=\frac{1}{p_1'p_2'p_3'\cdots p_r'}\,y_0. \tag{4}$$

If the equations had included n regions, the general formula for the number of tetrads of any class would be

$$X=\frac{1}{p_1'p_2'p_3'\cdots p_r'}\Bigg[b_0-\left(\frac{q_1}{p_1}b_1+\frac{q_2}{p_2}b_2+\cdots+\frac{q_n}{p_n}b_n\right)$$
$$+\left(\frac{q_1q_2}{p_1p_2}b_{12}+\frac{q_1q_3}{p_1p_3}b_{13}+\cdots+\frac{q_2q_3}{p_2p_3}b_{23}+\cdots+\frac{q_{(n-1)}q_n}{p_{(n-1)}p_n}b_{(n-1)n}\right)$$
$$+\cdots+(-1)^n\frac{q_1q_2q_3\cdots q_n}{p_1p_2p_3\cdots p_n}b_{123\cdots n}\Bigg]. \qquad (5)$$

Case 3. Recurrence not random, chance of detecting crossing over constant.

The frequencies of regressive, progressive, and digressive crossing over are not necessarily determined by chance alone: it is conceivable that they may vary with the nature of the crossings over (whether homologous or sister strand, lateral or diagonal), with their distance apart, and with the particular regions involved. They might also depend on other crossings over: their number, their distance away, the regions in which they occur, and their nature (including now not only whether they are homologous or sister-strand, and lateral or diagonal, but also whether they are regressive, progressive, or digressive with respect to the crossings over under consideration).

In making a table like tables 1 and 2, we may therefore subdivide each class of tetrad into its different types (such as are shown in figure 3) and distribute separately the chromatids emerging from each type.

If when we are considering tetrads of a given class we add together all the emerging strands that are crossovers in some specified k of the r regions, the proportion of such strands is no longer $p^r q^{r-k}$. The ratio of the actual proportion to the proportion expected on random recurrence may be designated by t; its value will in the most general case vary with the regions of crossing over in the tetrad and in the emerging chromatid; these regions may therefore be indicated by numerical subscripts, positive if the crossing over of the tetrad appears in the chromatid, negative if it does not. For example, the chance that a tetrad which is a crossover in regions 1, 2, and 3 will give rise to a chromatid which is a crossover in regions 1 and 3 only may be written as $t_{1-2+3}\,p^2\,q$.

In a table like table 1 the proper value of t will enter into the frequency of each class if recurrence is not random; for example, the distribution of tetrads of rank 3 will be as follows:

Regions of crossing over	0	1	2	3	12	13	23	123
Frequencies and distribution of tetrads of rank 3	$8t_{-1-2-3}$	$4t_{1-2-3}$	$4t_{-1+2-3}$	$4t_{-1-2+3}$	$2t_{1+2-3}$	$2t_{1-2+3}$	$2t_{-1+2+3}$	t_{1+2+3}

The x_0 values (the figures on the diagonal) for classes of rank 2 or more must be divided not merely by p^r but by the product of p^r and the appropriate t: the remainder in the 123 column must be divided by $t_{1+2+3}p^3$, that in the 23 column by $t_{2+3}p^2$, and so on.

The t's will also enter into the equations corresponding to those on page 163; this will be considered in connection with the next case. The general formula derived from the equations will now be more complicated; but if ranks above 2 are absent, the frequency of tetrads of rank 0 is

$$y_0 = a_0 - \frac{q}{p}a_1 + \frac{t_{1-2} + t_{-1+2} - t_{-1-2}}{t_{1+2}} \frac{q^2}{p^2} a_2. \tag{6}$$

Case 4. Recurrence not random, chance of detecting crossing over variable.

The same considerations apply to this case as to case 3 and the t factors enter into it in the same way. Thus the chance that a tetrad of rank 3 will give rise to a strand which is a recognizable crossover in regions 1 and 3 only will be $t_{1-2+3}p_1p_3q_2$.

With non-random recurrence, the distribution of tetrads of rank 3 in table 3 would be as follows:

Regions of crossing over	0	1	2	3	12	13	23	123
Frequencies and distribution of tetrads of rank 3	$2t_{-1-2-3}$	t_{1-2-3}	$2t_{-1+2-3}$	$2t_{-1-2+3}$	t_{1+2-3}	t_{1-2+3}	$2t_{-1+2+3}$	t_{1+2+3}

and each x_0 value must be divided by the product of $p_1'p_2' \cdots p_r'$ and the appropriate t.

The equations on page 166 will now become as follows.

$$\begin{aligned}
b_0 &= y_0 + q_1y_1 + q_2y_2 + q_3y_3 + t_{-1-2}q_1q_2y_{12} + t_{-1-3}q_1q_3y_{13} + t_{-2-3}q_2q_3y_{23} + t_{-1-2-3}q_1q_2q_3y_{123} \\
b_1 &= p_1y_1 + t_{1-2}p_1q_2y_{12} + t_{1-3}p_1q_3y_{13} + t_{1-2-3}p_1q_2q_3y_{123} \\
b_2 &= p_2y_2 + t_{-1+2}p_2q_1y_{12} + t_{2-3}p_2q_3y_{23} + t_{-1+2-3}p_2q_1q_3y_{123} \\
b_3 &= p_3y_3 + t_{-1+3}p_3q_1y_{13} + t_{-2+3}p_3q_2y_{23} + t_{-1-2+3}p_3q_1q_2y_{123} \\
b_{12} &= t_{1+2}p_1p_2y_{12} + t_{1+2-3}p_1p_2q_3y_{123} \\
b_{13} &= t_{1+3}p_1p_3y_{13} + t_{1-2+3}p_1p_3q_2y_{123} \\
b_{23} &= t_{2+3}p_2p_3y_{23} + t_{\;1+2+3}p_2p_3q_1y_{123} \\
b_{123} &= t_{1+2+3}p_1p_2p_3y_{123}
\end{aligned}$$

The formula for y_0 is now still more complicated; but if crossovers of ranks above 2 are absent or neglected,

$$\begin{aligned}
y_0 = b_0 - \left(\frac{q_1}{p_1}b_1 + \frac{q_2}{p_2}b_2 + \cdots + \frac{q_n}{p_n}b_n\right) \\
+ \frac{t_{1-2} + t_{-1+2} - t_{-1-2}}{t_{1+2}} \frac{q_1q_2}{p_1p_2} b_{12} + \frac{t_{1-3} + t_{-1+3} - t_{-1-3}}{t_{1+3}} \frac{q_1q_3}{p_1p_3} b_{13} \\
+ \cdots + \frac{t_{(n-1)-n} + t_{-(n-1)+n} - t_{-(n-1)-n}}{t_{(n-1)+n}} \frac{q_{(n-1)}q_n}{p_{(n-1)}p_n} b_{(n-1)n}.
\end{aligned} \tag{7}$$

In this formula, as in the equations from which it is derived, y_0 and b_0

represent respectively the number of tetrads and of chromatids of rank 0. If b_0 is used for a class of higher rank, the equations must be modified by omitting every term in which the y is not a crossover in at least the same regions as the b_0 class. The y's can then be renumbered so that the class of lowest rank is y_0, and a general formula can be derived as in cases 1 and 2. This, however, is complicated; and the frequencies of tetrads of classes ranking above 0 can be obtained by solving for the y's in the equations as they stand.

The frequency of chromatids

The observed frequency of any class includes of course only the chromatids that are homologous-strand crossovers in the specified regions. The true frequency of chromatids of the class would include sister-strand crossovers as well.

The relations between tetrad frequencies and true frequencies of chromatids are given by the sets of equations on pages 163, 166, and 168, and by equations 1–7 if the x's and y's retain their original meanings, but in the definitions of the a's, b's, p's, and t's the true frequencies of chromatids are substituted for the observed frequencies. The value of p will now vary from 1/2 when at any level of crossing over only two strands are involved to 1 when four strands are always involved in pairs.

The sets of equations on pages 163, 166, and 168, and equations 1–7 also express the relations between the observed and the true frequencies of chromatids if the a's and b's denote observed frequencies, the x's and y's the true frequencies of chromatids (not of tetrads), if each p denotes what proportion of exchanges are between homologous strands, and if the t's are modified accordingly (WEINSTEIN 1928, 1932a).

Each p as originally defined is of course the product of the p's of the two preceding paragraphs.

ORDINARY DIPLOIDS

The results of applying the multiple-strand method to ordinary diploids are illustrated in tables 4 and 8.

Table 4 is based on a cross involving almost the entire length of the X chromosome of *Drosophila melanogaster* (*sc ec cv ct v g f*). The experimental data (column A) comprise 28239 individuals, including 24034 from BRIDGES and OLBRYCHT (1926), 2047 from ANDERSON (1925a, table IV), and 2158 from an experiment (hitherto unpublished) by the writer. In the other columns are given tetrad frequencies calculated on the assumption of random recurrence for various values of p. The column headed $p = 1/2$ is based on the assumption that sister chromatids do not cross over (formula 2b); that headed $p = 1/3$ on the assumption that they cross over as freely

TABLE 4

Crossing over involving sc ec cv ct v g f (28239 individuals).

REGIONS OF CROSSING OVER	OBSERVED CHROMATID FREQUENCIES* p=2/3	TETRAD FREQUENCIES CALCULATED FOR RANDOM RECURRENCE							
		p=2/3	p=1/2	p=1/3 IN REGIONS INDICATED, 1/2 IN OTHER REGIONS					p=1/3
				1	12	123	1234	12345	
	A	B†	C	D	E	F	G	H	I
0	12776	6716	1709	837	−459	−2007	−4392	−5305	−5316
1	1407	1696	1744	2616	2601	2577	2220	1707	1203
2	2018	2483	2602	2592	3888	3864	3492	2724	1836
3	1976	2649	3130	3112	3096	4644	4527	4116	3546
4	3378	4515	5328	5094	4848	4770	7155	6861	6102
5	2356	2951	3180	2812	2296	2022	1826	2739	2610
6	2067	2284	1998	1626	1016	616	108	22	33
12	9	15	20	30	45	36	45	45	45
13	16	27	36	54	48	72	90	63	54
14	142	291	468	702	702	714	1071	981	846
15	198	430	736	1104	1104	1086	1026	1539	1530
16	206	440	744	1116	1110	1104	1014	1008	1512
23	11	21	36	32	48	72	81	81	99
24	136	291	492	492	738	744	1116	1098	1008
25	261	584	1032	1032	1548	1548	1536	2304	2295
26	318	701	1224	1220	1830	1842	1782	1776	2664
34	42	88	148	152	156	234	351	351	306
35	148	324	560	548	548	822	822	1233	1188
36	212	463	800	792	800	1200	1170	1140	1710
45	123	262	440	400	392	392	588	882	873
46	315	674	1136	1076	1036	1016	1524	1518	2277
56	59	124	204	200	196	176	172	258	387
123	3	7	8	12	18	27			−27
124	1	2				−18	−27	−27	−27
126	2	5	8	12	18				
134		−2	−8	−12	−24	−36	−54	−54	−54
135	3	10	24	36	36	54	54	81	81
136	3	8	16	24	12	18	18	18	27
145	10	34	80	120	120	120	180	270	270
146	15	51	120	180	180	180	270	270	405
156	1	3	8	12	12	12	12	18	27
234	1	2		−8	−12	−18	−27	−27	−27
236		−2	−8	−16	−24	−36	−36	−36	−54
245	2	7	16	16	24	24	36	54	54
246	10	34	80	80	120	120	180	180	270
256	1	3	8	8	12	12	12	18	27
346	5	17	40	40	40	60	90	90	135
356	5	17	40	40	40	60	60	90	135
456	1	3	8	8	8	8	12	18	27
1234	1	5	16	24	36	54	81	81	81
1236	1	5	16	24	36	54	54	54	81

† In the p=2/3 column, the only one in which fractional values occur, they are given to the nearest unit. The values in the p=2/3 column also represent the true frequencies of chromatids when p=1/3.

* From BRIDGES and OLBRYCHT 1926, ANDERSON 1925a, and WEINSTEIN.

as other chromatids (formula 2a). In the latter column the frequency of the non-crossover class is negative; and since this is impossible, it follows that there cannot be random association of chromatids with free crossing over between sister strands.

The results are of course subject to errors of sampling as well as errors due to differential viability and other causes. These errors may become exaggerated in the course of the calculations because the observed frequencies of the crossovers of higher rank are small and are multiplied by relatively large factors, so that slight differences may be magnified. For this reason the negative frequencies among the crossovers (they are all among the triples) are obviously not significant, for they would become 0 or positive with small changes in the observed numbers of triples or quadruples, or in some cases if the quadruples were derived from tetrads of rank 5.

The errors of sampling may be calculated by the formula for the standard error of a function of several variables, which may be written

$$E=\sqrt{\left(\frac{\partial F}{\partial v_0}\right)^2 e_0{}^2+\left(\frac{\partial F}{\partial v_1}\right)^2 e_1{}^2+\left(\frac{\partial F}{\partial v_2}\right)^2 e_2{}^2+\cdots+\left(\frac{\partial F}{\partial v_n}\right)^2 e_n{}^2}$$

where F is a function of the v's, E its standard error, and the e's are the standard errors of the respective v's. This formula holds for all cases where the distribution of errors is Gaussian (SCARBOROUGH 1930, pp. 337–338). For F we may substitute the tetrad frequency X; and for the v's we may substitute the a's of formula 2 or 2a or 2b, or the b's of formula 5.

The frequency of the non-crossovers in the $p=1/3$ column is approximately 20 times its standard error, which is 268. The result cannot therefore be due to errors of sampling. Nor can it be due to differential viability, for viability was good in the experiments on which the calculations are based.

We may conclude that sister strands do not cross over as freely as homologous strands if the association of chromatids in crossing over is otherwise random. It does not follow however that they do not cross over at all: they might cross over only in some regions, or throughout the chromosome but to a smaller extent than homologous strands.

To test the first of these alternatives, tetrad frequencies were calculated by means of formula 5 for $p=1/3$ (free sister-strand crossing over) in some regions and $p=1/2$ (no sister-strand crossing over) in others. The results are given in columns D–H of table 4. As sister-strand crossing over is restricted to a shorter and shorter region at the left of the X chromosome, the negative frequencies approach 0 and finally become positive. The negative frequencies are from about 10 to about 20 times their standard errors, except -459, which is about 2.5 times its standard error. Thus it is shown that free crossing over between sister chromatids, if it occurs at all, must be limited to a short region. This is not necessarily at the left end of the X, for the results are similar if we postulate sister-strand crossing over in other regions.

There remains the possibility that recurrence is not random. This can be tested by seeing whether the relative frequencies of tetrads can be altered without changing the frequencies of strands as given by experiment.

TABLE 5

Frequencies of tetrads of ranks 0, 1, and 2 and of strands derived from them, on the assumption of random association without sister-strand crossing over ($p = 1/2$).

RANK AND TYPE OF TETRAD	FREQUENCY OF TETRAD	FREQUENCIES OF STRANDS		
		NON-CROSSOVERS	SINGLES	DOUBLES
0	1709	1709		
1	17982	1/2=8991	1/2=8991	
2				
1/4=regressives	2019	1/2=1009.5		1/2=1009.5
1/2=progressives	4038	1/4=1009.5	1/2=2019	1/4=1009.5
1/4=digressives	2019		2019	

Table 5 gives the frequencies of tetrads of ranks 0, 1, and 2 and of strands derived from them on the assumption of random association without sister-strand crossing over. The following equations indicate what combinations of tetrads are equivalent with respect to the strands derived from them:

1 non-crossover +1 digressive = 2 singles. (8)

1 regressive +1 digressive = 2 progressives. (9)

1 non-crossover +2 progressives = 1 regressive +2 singles. (10)

Still other substitutions are possible if ranks above 2 are included; for example,

1 single +1 regressive = 1 non-crossover +1 digressive (or regressive-digressive) of rank 3. (11)

By a rank-3 digressive is meant a tetrad like D1 in figure 3; by a rank-3 regressive-digressive is meant one like D2 or B2.

In making these substitutions, it is necessary to consider the regions of crossing over, so that the proper classes and frequencies of strands may result; also in order to avoid digressives with two crossings over in the same region, unless it is desired to test the possibility of such digressives. The substitutions are also limited by the frequencies of tetrads to be replaced; hence it follows that deviations from random recurrence will, if too great, lead to negative frequencies of tetrads. Nevertheless considerable deviations are possible without changes in the frequencies of strands;

but coincidence of tetrads will be altered, as will also the frequencies of progeny of attached X's. These results will be considered below.

Equations 8 and 11 hold when sister strands cross over freely; the other equations now assume the following forms:

$$1 \text{ regressive} + 3 \text{ digressives} = 4 \text{ progressives.} \qquad (12)$$

$$3 \text{ non-crossovers} + 4 \text{ progressives} = 1 \text{ regressive} + 6 \text{ singles} \qquad (13)$$

If sister strands cross over freely, then as can be seen from table 6, random recurrence results in an excess of tetrads of ranks higher than 0 and of non-crossover strands derived from them, so that a negative frequency of tetrads of rank 0 must be postulated to bring the total number of tetrads and of non-crossover strands down to the observed figure. The negative frequency will disappear if non-random recurrence can reduce by 5316 the number of those tetrads of ranks higher than 0 that give rise to non-crossover strands. The simplest way to do this is to replace 10632 tetrads of rank 1 by their equivalent 5316 non-crossover tetrads and 5316 digressives, in accordance with equation 8.

TABLE 6

Frequencies of tetrads of ranks 0, 1, and 2 and of strands derived from them on the assumption of random association including sister-strand crossing over ($p=1/3$).

RANK AND TYPE OF TETRAD	FREQUENCY OF TETRAD	FREQUENCIES OF STRANDS NON-CROSSOVERS	 SINGLES	 DOUBLES
0	−5316	−5316		
1	15330	2/3 = 10220	1/3 = 5110	
2				
1/6 = regressive	2799	2/3 = 1866		1/3 = 933
2/3 = progressive	11196	5/12 = 4665	1/2 = 5598	1/12 = 933
1/6 = digressive	2799	1/3 = 933	2/3 = 1866	

TABLE 7

Frequencies of tetrads of ranks 0, 1, and 2 and of strands derived from them on the assumption of non-random association with sister strand crossing over ($p=1/3$). (For explanation see text.)

RANK AND TYPE OF TETRAD	FREQUENCY OF TETRAD	FREQUENCIES OF STRANDS NON-CROSSOVERS	 SINGLES	 DOUBLES
0	0	0		
1	4698	3132	1566	
2				
regressive	2799	1866		933
progressive	11196	4665	5598	933
digressive	8115	2705	5410	

The tetrad and strand frequencies as revised by this method are given in Table 7. They may be modified by other substitutions.

TABLE 8

Crossing over involving sc ec cv ct v s f car bb (16136 individuals).

REGIONS OF CROSSING OVER	OBSERVED CHROMATIN FREQUENCIES*	TETRAD FREQUENCIES CALCULATED FOR RANDOM RECURRENCE									
		p=2/3	p=1/2	p=1/3 in regions indicated, 1/2 in other regions							p=1/3
				1	12	123	1234	12345	123456	1234567	
	A	B†	C	D	E	F	G	H	I	J	K
0	6607	3300	904	697	175	−301	−1161	−1547	−1683	−1462	−1339
1	506	530	414	621	618	609	501	357	18	−120	−204
2	1049	1167	1046	1044	1566	1566	1497	1266	624	327	189
3	855	996	960	952	952	1428	1371	1230	717	483	399
4	1499	1817	1876	1804	1758	1720	2580	2466	1914	1494	1305
5	937	1143	1196	1096	942	848	772	1158	1080	981	903
6	1647	1867	1710	1480	1036	692	324	272	408	363	333
7	683	661	420	320	100	−66	−346	−412	−442	−663	−651
8	379	350	202	140	24	−42	−180	−234	−254	−246	−369
12	3	4	4	6	9	0	0	0	−9	−9	−9
13	6	11	16	24	18	27	27	18	18	18	18
14	41	87	144	216	216	216	324	315	306	288	279
15	55	118	200	300	300	294	288	432	432	414	405
16	118	262	460	690	684	684	678	678	1017	1017	1008
17	54	117	200	300	300	300	288	276	276	414	414
18	34	73	124	186	186	186	180	174	168	168	252
23	3	4	4	0	0	0	0	0	−9	−9	−9
24	38	69	92	92	138	138	207	207	171	126	72
25	85	182	308	308	462	462	462	693	666	630	621
26	237	517	892	888	1332	1326	1302	1284	1926	1908	1881
27	123	262	440	440	660	660	630	606	594	891	873
28	70	144	232	232	348	348	312	306	288	276	414
34	22	46	76	76	76	114	171	171	171	153	144
35	55	116	192	188	188	282	282	423	414	396	369
36	177	394	692	692	688	1032	1032	1026	1539	1530	1521
37	88	192	332	332	332	498	486	474	468	702	702
38	38	80	132	132	132	198	192	174	168	168	252
45	41	90	156	152	152	152	228	342	342	342	333
46	198	435	756	752	736	736	1104	1104	1656	1656	1620
47	159	346	596	588	568	560	840	840	840	1260	1251
48	91	189	308	304	280	276	414	408	384	378	567
56	35	73	120	120	108	104	104	156	234	234	225
57	49	101	164	156	140	132	132	198	198	297	297
58	40	82	132	128	124	112	108	162	156	156	234
67	21	44	72	72	64	60	60	60	90	135	135
68	30	56	80	76	64	60	44	40	60	60	90
78	2	1	−4	−4	−12	−12	−16	−16	−16	−24	−36

* From BRIDGES.

† See footnote to table 4.

TABLE 8 (*Continued*)

Crossing over involving sc ec cv ct v s f car bb (16136 individuals).

	A	B	C	D	E	F	G	H	I	J	K
123	1	3	8	12	18	27	27	27	27	27	27
126	1	3	8	12	18	18	18	18	27	27	27
135	1	3	8	12	12	18	18	27	27	27	27
145	1	3	8	12	12	12	18	27	27	27	27
146	1	3	8	12	12	12	18	18	27	27	27
147	2	7	16	24	24	24	36	36	36	54	54
148	1	3	8	12	12	12	18	18	18	18	27
157	2	7	16	24	24	24	24	36	36	54	54
158	1	3	8	12	12	12	12	18	18	18	27
168	1	3	8	12	12	12	12	12	18	18	27
236	1	3	8	8	12	18	18	18	27	27	27
246	4	13	32	32	48	48	72	72	108	108	108
247	5	17	40	40	60	60	90	90	90	135	135
248	6	20	48	48	72	72	108	108	108	108	162
256	3	10	24	24	36	36	36	54	81	81	81
257	4	13	32	32	48	48	48	72	72	108	108
258	1	3	8	8	12	12	12	18	18	18	27
267	2	7	16	16	24	24	24	24	36	54	54
268	3	10	24	24	36	36	36	36	54	54	81
278	2	7	16	16	24	24	24	24	24	36	54
347	2	7	16	16	16	24	36	36	36	54	54
348	1	3	8	8	8	12	18	18	18	18	27
356	1	3	8	8	8	12	12	18	27	27	27
357	2	7	16	16	16	24	24	36	36	54	54
358	3	10	24	24	24	36	36	54	54	54	81
367	1	3	8	8	8	12	12	12	18	27	27
368	1	3	8	8	8	12	12	12	18	18	27
458	1	3	8	8	8	8	12	18	18	18	27
468	4	13	32	32	32	32	48	48	72	72	108
478	1	3	8	8	8	8	12	12	12	18	27
568	1	3	8	8	8	8	8	12	18	18	27

In the above analysis it has been assumed that while the relative frequencies of tetrads may differ, within each type of tetrad the frequencies of strands that are recognizable as crossovers in any specified regions are the same as when recurrence is random. This is necessarily true when sister strands do not cross over, but otherwise need not be true for all tetrads. Hence substitutions differing from those given above might be possible.

The results of non-random recurrence can also be tested by means of the formulas for cases 3 and 4. For the frequency of tetrads of rank 0, formula 6 will be sufficient, at any rate for purposes of illustration.

An increase of regressives, or of progressives at the expense of digressives, will decrease t_{1-2} and t_{-1+2} and will increase t_{1+2} and t_{-1-2}. This will lessen the value of the term in a_2 and consequently of y_0; hence there will be even more negative tetrads if sister strands cross over. An increase of digressives, or of progressives at the expense of regressives, will increase t_{1-2} and t_{-1+2} and will decrease t_{1+2} and t_{-1-2}. This will increase the value of the term in a_2 and if the increase is sufficient the negative frequency of non-crossover tetrads will disappear.

Other classes can be evaluated in the same way; and the method is essentially equivalent to the analysis already given. For some purposes it may be preferable, since it can be used more systematically; for example, the t's may be made to vary according to some rule, such as that $t_{1+2+3} = t_{1+2}t_{2+3}$, and the correctness of the rule can thus be tested.

The analysis of the Xple data is supported by the results of applying the multiple-strand theory to a cross involving the genes *sc ec cv ct v s f car bb*, which cover practically the entire crossover map of the X chromosome (table 8). The observed frequencies (column A) are from an experiment made by DR. C. B. BRIDGES, who kindly placed them at my disposal before they were published (MORGAN, BRIDGES, and SCHULTZ 1935).

Negative values appear in the non-crossover tetrads as well as some of those of rank 1 when sister strands are allowed to cross over beyond the first two regions.

Here the negative values cannot be eliminated so simply as in the X-ple cross.

The analysis is further supported by the application of the theory to other data, both sex-linked and autosomal, in *Drosophila melanogaster* and to sex-linked data in *D. virilis*. These results will be published elsewhere.

The maximum amount of recombination between two linked genes

In most organisms the amount of recombination between two linked genes approaches 50 percent as an upper limit as the intermediate distance lengthens. This relation is a corollary of equation 2b, which can be written

$$(a_0+a_2+a_4+\cdots)-(a_1+a_3+a_5+\cdots)=2^rX.$$

If the assumptions on which the equation is based are correct, then 2^rX cannot be negative. Hence $(a_0+a_2+a_4+\cdots)\geqq(a_1+a_3+a_5+\cdots)$; that is, the apparent non-crossovers (classes of 0 and even rank) will equal or exceed the apparent crossovers (classes of odd rank).

This relation can be deduced directly from the set of equations on page 163; as in fact it has been deduced by EMERSON and RHOADES (1933) from the table of BELLING (1931) which corresponds to a special case of these equations. In each vertical column of the equations the coefficients of the x's are the successive terms of the expansion $(p+q)^r$, where $q=1-p$. If $p=1/2$, the sum of the alternate terms is 1/2; and in any column (except the x_0 column) the strands with no crossings over or with an even number will constitute half the total. These strands together with those in the x_0 column make up the apparent non-crossovers; hence the observed recombination frequency will be less than 50 percent. As the chromosome lengthens genetically, the x_0 class will decrease and approach 0 and the proportion of recombination between the end genes will approach 50 percent as an upper limit.

A more general relation can be obtained if the equations are multiplied respectively by $1, -1, 1, \cdots (-1)^n$ and added together. The result is the equation

$$(a_0+a_2+a_4+\cdots)-(a_1+a_3+a_5+\cdots)$$
$$=x_0+(q-p)x_1+(q-p)^2x_2+\cdots+(q-p)^nx_n. \qquad (14)$$

If $p=1/2$, $q-p=0$, and we have the case just discussed.

If $p<1/2$, the right-hand side of the equation will remain greater than 0, and the recombination frequency between the end genes will remain less than 50 percent as n increases.

If $p>1/2$, the terms in $(q-p)$ will be positive if the exponent is even, negative if it is odd. Hence their sum will be increased by the terms with even exponents, decreased by those with odd exponents. The precise nature of the result will therefore depend on the number of x's and their relative sizes; that is, on the length of the chromosome and the coincidence. This situation would result if more than two chromatids crossed over at one level or within a region.

The relations just deduced are only part of still more general relations. For in the equations on page 163, a_0 may represent the frequency of any class, not necessarily the non-crossovers. It follows that if $p=1/2$, the sum of the crossovers in any specified region or regions and in 0, 2, 4, · · · additional regions will exceed the sum of the crossovers in the specified region or regions and in 1, 3, 5, · · additional regions. This relation holds for *Drosophila melanogaster* and also for *D. virilis* (WEINSTEIN, unpublished data involving the X chromosome from sepia to rugose, a distance of about 100 units.

The minimum distance within which double crossing over occurs

An upper limit can be set to the possible frequency of crossing over between sister strands by the length of the shortest distance between adjacent levels of crossing over in a chromatid. In the X chromosome of *D. melanogaster*, this is about 14 units. Since the frequency of tetrad crossing over in such a region cannot exceed 1.00, the chance of detecting a crossing over, being the ratio of the observed to the actual frequency, cannot be less than 0.14. But the chance of detecting a crossing over in a tetrad is composed of two factors: the chance of recovering a crossover chromosome and the chance of recognizing it when recovered. The first factor is 1/2 (provided that only two strands cross over within the region); hence the second is not less than 0.28. That is, crossover strands can be recognized in at least 0.28 of cases, and sister strands cannot cross over in more than 0.72 of cases.

It might be possible to draw a further conclusion in some cases. If every tetrad were a crossover in the region, the amount of crossing over in the region could not exceed 33 1/3 percent if sister strands crossed over and 50 percent if they did not. Thus single crossing over exceeding 33 1/3 percent within a region where no double crossing over occurred might be an indication that there was no crossing over between sister strands.

COINCIDENCE AND INTERFERENCE

The coincidence of two regions may be expressed as

$$\frac{D/N}{A/N \quad B/N} = \frac{DN}{AB},$$

where N is the total number of individuals, A the number of crossovers in the first region, B in the second region, and D in both regions simultaneously. The regions whose coincidence is being measured will be referred to as nodal regions; the points of crossing over as nodes. The distance between the nodes will be termed the internode, and the distance between the nodal regions will be termed the intermediate region.

Coincidence may be of several types. In the type originally defined, A, B, and D include all individuals that are crossovers in the nodal regions, regardless of whether they are also crossovers in other regions. They may be termed inclusive totals, and coincidence so calculated inclusive coincidence. Inclusive coincidence, which is a measure of interference, increases as the internode lengthens (MULLER 1916).

If crossovers in the intermediate region are excluded from D, the resultant coincidence may be termed select; it measures the frequencies of internodes of different lengths (WEINSTEIN 1918).

If crossovers in the intermediate region are excluded from A, B, D, and N, the resultant coincidence has been termed partial (MULLER 1925). The concept of partial coincidence may be extended by excluding regions other than the intermediate ones; in the extreme case all regions may be excluded except those whose coincidence is being measured. This extreme type may be termed exclusive coincidence.

All the above types of coincidence as ordinarily measured are based on the observed frequencies of chromatids. They may therefore be termed chromatid or strand coincidence. Coincidence might also be based on true chromatid frequencies, or on tetrad frequencies. Tetrad coincidence might be based merely on the levels of crossing over, regardless of which strands are involved; or it might be calculated separately for regressive, progressive, and digressive crossing over; and further distinctions are possible according to whether the exchanges are between lateral or diagonal, homologous or sister strands.

Inclusive coincidence

If recurrence is random, inclusive coincidence for tetrads or for the true frequencies of strands is equal to the strand coincidence as ordinarily calculated. For if X_A, X_B and X_D denote respectively the inclusive frequencies of tetrads that are crossovers in the first nodal region, in the second nodal region, and in both nodal regions, then strand coincidence whether based on observed or true values is $p_A\ p_B\ X_D\ N/p_A\ X_A\ p_B\ X_B = X_D\ N/X_A\ X_B$, which is the tetrad coincidence. This invariance holds regardless of whether or not sister strands cross over, and regardless of whether the p's are the same or different in different regions.

But if recurrence is not random, strand coincidence is

$$t_{A+B}\ p_A\ p_B\ X_D\ N/p_A\ X_A\ p_B\ X_B;$$

hence

$$\text{inclusive tetrad coincidence} = \frac{\text{inclusive chromatid coincidence}}{t_{A+B}} \qquad (15)$$

If random recurrence is altered by an increase of progressives at the expense of digressives, or by an increase of regressives, $t_{A+B} > 1$, and tetrad coincidence will be less than the observed value. If random recurrence is altered by an increase of progressives at the expense of regressives, or by an increase of digressives, $t_{A+B} < 1$, and tetrad coincidence will be greater than the observed value.

Only those deviations from random recurrence need be considered that yield the observed frequencies of chromatids: for example, those indicated in equations 8–13 and in table 7. These deviations will not alter the frequency of recognizable crossing over in any one region, and hence will not alter the denominator of the coincidence fraction. Their effect on coincidence will result from an alteration of the number of double crossover tetrads and hence of the numerator of the fraction.

The substitutions indicated in equations 9, 11, and 12 would not alter the total number of tetrads that are crossovers in both regions involved and hence would not affect the coincidence. This applies to equation 11 only if the rank-2 regressive involves both nodal regions. But a change would result from the substitutions indicated in the other equations. Since, on random recurrence without sister-strand crossing over, the progressives constitute one-half of rank-2 tetrads, the regressives and digressives one quarter each, the substitutions in equation 10 might increase or decrease tetrad coincidence by as much as 25 percent. A similar decrease might result from the elimination of digressives indicated in equation 8. The reverse substitution in this equation would increase tetrad coincidence by an amount dependent on the number of singles available. Thus the coincidence of regions 1 and 6, which is $912 \cdot 28239/4036 \cdot 6442 = 1.0$, could be increased to 2.9 if all the 1744 singles in region 1 and an equal number in region 6 were replaced by 1744 digressives and 1744 non-crossovers. Similarly, the coincidence of regions 2 and 6 could be increased from 1.0 to 2.5 if the 1998 singles in region 6 and an equal number in region 2 were replaced by digressives and non-crossovers.

Not all the coincidence values could be altered simultaneously. Thus if all the 1998 singles in region 6 were used to raise the 2, 6 coincidence, there would be none left to raise the coincidence of 1 and 6. Again, only 1709 digressives could be added or eliminated by equation 8 since there are only 1709 non-crossovers. If the changes are distributed among the various classes, the increase or decrease in any one class would be much less than indicated above.

Similar procedures with similar results apply if there is crossing over between sister strands. The distribution of tetrads in table 7 would increase coincidence since it adds 5316 tetrads to rank 2. The increase would be negligible for all values involving region 6, for the additional doubles are at the expense of singles, of which there are only 33 in region 6 (table 4, column I); but other values might be considerably affected. The increases could be minimized if the additional tetrads were distributed among all classes; but they could not be counteracted by substitutions since there are no non-crossovers at whose expense these could be made.

It is perhaps unlikely that inclusive tetrad coincidence differs greatly from the observed value, for the following reasons: (1) Great deviations are brought about only by restricting substitutions of tetrads to one or a few classes, with the result that two adjacent regions have very different coincidences with the same region. (2) An increase of coincidence to more than 1 would imply that crossing over in one region is helped by that in another. (3) A decrease of inclusive coincidence for widely separated regions to less than 1 is not consistent with the mutual independence that might be expected from such regions. But we do not know how widely separated two regions must be to achieve independence; and in our present state of knowledge none of these reasons is conclusive.

Select coincidence

If $X_d, X_{d+1}, X_{d+2}, \cdots X_{d+n}$ are the frequencies of tetrads that are crossovers in both nodal regions and also at 0, 1, 2, $\cdots$ n levels in the intermediate region, then if recurrence is random and p constant,

$$\text{select chromatid coincidence} = \frac{p^2[X_d + qX_{d+1} + q^2X_{d+2} + \cdots + q^nX_{d+n}]N}{p\,X_A \quad p\,X_B}$$

$$= \text{select tetrad coincidence} + \frac{[qX_{d+1} + q^2X_{d+2} + \cdots + q^nX_{d+n}]N}{X_A\,X_B} \qquad (16)$$

Hence select coincidence for tetrads is less than the true value for chromatids, which in turn is less than the observed value since q for the true value is less than for the observed value. These relations hold if the p's differ, but not necessarily if recurrence is not random.

As the intermediate region lengthens, there will ultimately be a decrease in the frequency of tetrads that are not crossovers in it, and consequently a decrease of select tetrad coincidence. Select coincidence for strands will also ultimately decrease if recurrence is random, and even if it is not random except on rather special assumptions.

Partial coincidence

Partial coincidence for tetrads will in general differ from the observed value; for when tetrad frequencies are replaced by the corresponding strand frequencies, the changes in the numerator and denominator do not necessarily compensate for each other. The same is true when non-intermediate regions or all non-modal regions are excluded.

A detailed discussion of coincidence in *D. melanogaster* and *D. virilis* will be published separately.

NON-DISJUNCTION AND ATTACHED X'S

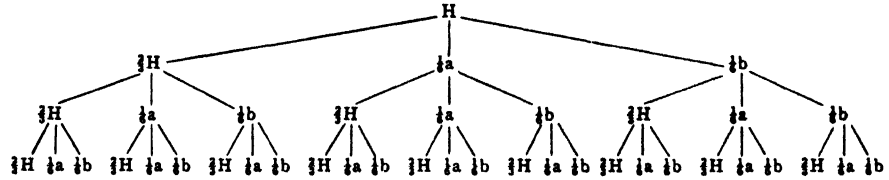
The multiple-strand method can be applied to cases of non-disjunction and attached X's if allowance is made for the fact that two chromatids are recovered instead of one and that they are not necessarily a random pair. The situation is clearest in attached X's, where the genes in the attached strands tend to remain together, the tendency being absolute at the point of attachment and decreasing distally because of crossing over with strands of the other attached pair.

It will be best therefore to begin at the point of attachment; and if we do this, the strands resulting from tetrads of any given rank can be deduced as follows.

Crossing over between the spindle fibre and the first heterozygous pair of genes might occur in three ways: (1) between strands attached to each other (these are homologous); (2) between sister strands; and (3) between a strand and its homologue in the other attached pair. Types 1 and 2 will leave each attached pair heterozygous; type 3 will result in homozygosis in each pair. If any two chromatids are equally likely to cross over, the three types will occur with equal frequency, and homozygosis will be produced in 1/3 of the cases.

If the first crossing over leaves each attached pair of chromatids heterozygous, then crossing over in the next region can occur in the same three ways, with the same results. If the first crossing over has produced homozygosis, the next crossing over can occur in two ways: (1) between strands attached to each other (these are now sister strands), (2) between strands of different attached pairs (these are now all homologous). If it is a matter of chance which strands cross over, type 1 will occur in 1/3 of cases, type 2 in 2/3.

This procedure can be continued for the entire length of the chromosome. It may be represented by the following diagram, in which a represents homozygosis for one strand, b for the other, and H heterozygosis.


H
⅔H ⅙a ⅙b
⅔H ⅙a ⅙b ⅔H ⅙a ⅙b ⅔H ⅙a ⅙b
⅔H ⅙a ⅙b ⅔H ⅙a ⅙b ⅔H ⅙a ⅙b ⅔H ⅙a ⅙b ⅔H ⅙a ⅙b ⅔H ⅙a ⅙b ⅔H ⅙a ⅙b ⅔H ⅙a ⅙b ⅔H ⅙a ⅙b

The chance that a tetrad of any rank will give rise to a particular type of offspring can be obtained by multiplying the appropriate fractions. For example, among offspring derived from tetrads of rank 3, there will be $(2/3)^3$ that are heterozygous throughout and $(1/6)^3$ that are homozygous for strand a throughout.

If crossing over does not occur between sister strands, but association of strands is otherwise random, the procedure must be modified to conform with the following diagram:

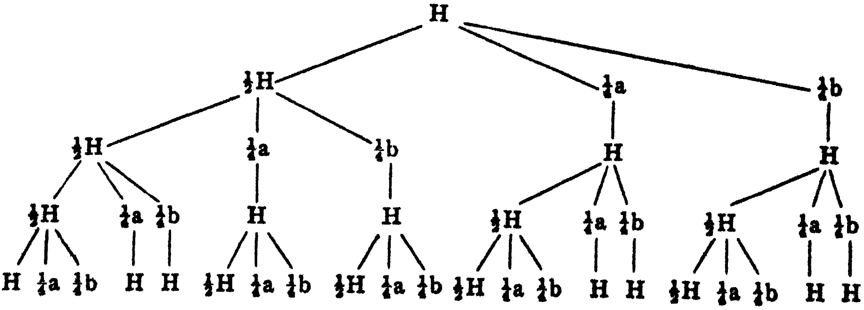

H
½H ¼a ¼b
½H ¼a ¼b H H
½H ¼a ¼b H H ½H ¼a ¼b ½H ¼a ¼b
½H ¼a ¼b H H ½H ¼a ¼b ½H ¼a ¼b ½H ¼a ¼b H H ½H ¼a ¼b H H

TABLE 9*

Calculated and observed frequencies of daughters of attached-X females
(Observed frequencies from Sturtevant 1931)

I $\frac{+}{sc\ ec\ cv\ ct\ v\ g\ f}$

	Obs.	Calc.
+	614	613
sc	27	19
sc ec	36	29
sc ec cv	18	27
sc ec cv ct	47	45
sc ec cv ct v	16	25
sc ec cv ct v g	9	15
sc ec cv ct v g f	5	9
ec	1	0.2
ec cv	2	0.4
ec cv ct	4	4
ec cv ct v	5	6
ec cv ct v g	9	6
ec cv ct v g f	3	6
cv	2	0.4
cv ct	7	4
cv ct v	12	8
cv ct v g	10	9
cv ct v g f	3	6
ct	4	1
ct v	4	5
ct v g	6	6
ct v g f	5	6
v	6	3
v g	8	10
v g f	9	7
g	1	2
g f	4	4
f	1	1
sc v g	2	0.2
Total	880	

II $\frac{ec\ cv\ ct\ v\ g\ f}{sc}$

	Obs.	Calc.
+	557	578
sc	198	210
ec	34	36
ec cv	29	35
ec cv ct	54	61
ec cv ct v	32	38
ec cv ct v g	24	26
ec cv ct v g f	6	19
cv ct	9	5
cv ct v	19	10
cv ct v g	7	12
cv ct v g f	8	8
ct	0	1.6
ct v	15	6
ct v g	10	8
ct v g f	7	8
v	9	4
v g	18	12
v g f	21	9
g	3	2
g f	17	4
f	2	1.1
ec v g	1	0.2
ec cv g f	2	0.1
ec cv ct g f	2	0.5
ec cv ct f	1	0.2
sc v g	1	0.6
sc v g f	3	0.3
sc g	1	0.2
sc g f	4	0.7
sc f	1	0.2
sc ct v g f	1	0
Total	1096	

III $\frac{g\ f}{sc\ ec\ cv\ ct\ v}$

	Obs.	Calc.
+	515	534
sc	16	19
sc ec	20	28
sc ec cv	22	27
sc ec cv ct	28	44
sc ec cv ct v	39	48
g	56	49
g f	38	32
ec cv	2	0.4
ec cv ct	7	4
ec cv ct v	28	17
cv	1	0.4
cv ct	5	4
cv ct v	28	23
ct	2	1.3
ct v	30	17
v	27	20
f	2	1.1
sc v	2	0.5
sc ec v	1	0.2
sc g	1	0.3
sc ec g f	3	0.2
Total	873	

IV $\frac{ec\ cv\quad\quad g\ f}{sc\quad\quad ct\ v}$

	Obs.	Calc.
+	308	318
sc	74	61
sc ct	39	37
sc ct v	36	40
ec	28	46
ec cv	83	65
ec cv g	9	18
ec cv g f	5	13
ct	17	8
ct v	37	48
cv	6	10
cv g	7	8
cv g f	4	5
g	21	15
g f	17	14
v	25	16
f	2	0.7
ec g	1	0.1
ec g f	1	0.2
ec cv f	1	0.1
sc g	1	0.5
sc g f	1	0.5
sc f	1	0.1
sc ct g	1	0.0
Total	725	

V $\frac{ct\ v\quad f}{sc\ ec\ cv\quad\quad g}$

	Obs.	Calc.
+	349	362
sc	22	16
sc ec	15	23
sc ec cv	56	80
sc ec cv g	11	21
ct	46	44
ct v	68	66
ct v f	15	23
ec cv	17	8
ec cv g	10	10
cv	14	10
cv g	15	13
v	15	11
v f	7	6
g	44	29
f	14	4
sc g	3	0.5
sc ec g	2	0.3
sc ct v	1	0.1
sc v f	1	0.2
sc f	1	0.1
sc ec v f	1	0.1
sc ec f	1	0.2
Total	728	

VI $\frac{ec\quad\quad v\quad f}{sc\quad cv\ ct\quad g}$

	Obs.	Calc.
+	386	397
sc	62	49
sc cv	20	28
sc cv ct	37	72
sc cv ct g	9	26
ec	102	109
ec v	43	54
ec v f	13	16
cv	2	0.8
cv ct	32	22
cv ct g	28	29
ct	14	6
ct g	12	13
v	61	43
v f	24	20
g	39	23
f	11	4
cv g	1	0
sc g	5	1
sc v	6	0.6
sc v f	4	0.3
sc f	1	0.3
ec g	4	1
Total	916	

VII $\frac{ec\quad ct\ v\quad f}{sc\quad cv\quad\quad g}$

	Obs.	Calc.
+	352	398
sc	69	49
sc cv	90	100
sc cv g	12	26
ec	65	58
ec ct	48	50
ec ct v	54	53
ec ct v f	4	16
cv	31	23
cv g	34	29
ct	14	6
ct v	44	29
ct v f	15	13
v	15	14
v f	5	7
g	47	36
f	3	4
sc g	2	1
ec v	3	0.3
ec ct f	1	0.5
sc ct v	1	0.0
sc v f	2	0.3
sc f	1	0.3
ec g	2	0.6
ec ct g	1	0.4
ec cv	1	0
Total	916	

VIII $\frac{cv\quad\quad g}{sc\ ec\quad ct\ v\quad f}$

	Obs.	Calc.
+	413	433
sc	35	20
sc ec	59	59
sc ec ct	38	47
sc ec ct v	21	42
sc ec ct v f	2	9
cv	121	125
cv g	40	55
ec	8	0.6
ec ct	7	4
ec ct v	17	12
ec ct v f	3	6
ct	10	6
ct v	41	30
ct v f	21	13
g	43	37
v	17	14
v f	19	7
f	4	5
sc v f	1	0.2
sc ec v	1	0.4
sc cv g	1	0.1
sc g	1	0.6
sc ec g	3	0.6
sc ec ct g	1	0.3
ec g	1	0.0
Total	928	

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* This table includes all calculated frequencies of 1 or more; and, where the observed frequency is not 0, every calculated frequency of less than 1. Calculated figures are given to the nearest unit; except those of 1 or less, which are given to the nearest 0.1.

In either of the above cases, if recurrence is not random, the frequency of each class obtained from a tetrad of a given kind will not be the simple product of the fractions in the table, but this product multiplied by a factor which, in the most general case, will differ according to the rank of the tetrad, the regions involved, and the nature of the crossings over: whether homologous or sister-strand; lateral or diagonal; regressive, progressive, or digressive.

Thus if the frequencies of tetrads are known, the frequencies of genotypes and phenotypes among the offspring can be calculated. In table 9 are given the frequencies of phenotypes expected among offspring of attached-X females heterozygous for *sc ec cv ct v g f*, on the assumption that sister strands do not cross over but association of chromatids is otherwise random. The tetrad frequencies in the column headed $p=1/2$ in table 4 have been used as a basis; but since they do not include crossing over between the spindle fibre and forked, a correction has been applied by taking into account what proportion of each class must also have been crossovers to the right of forked. This correction is based on a cross involving the loci *y bi cv ct v g B bb*, the unpublished data of which were kindly placed at my disposal by DR. C. B. BRIDGES in 1932. The crossover values in this cross agree closely with those in the X-ple cross (MORGAN, BRIDGES and SCHULTZ 1933).

The calculations have been made for the eight types of heterozygous mothers that gave the greatest number of offspring in the experiments of STURTEVANT (1931), and STURTEVANT's actual counts are included for comparison. The frequencies are on the whole in agreement. The discrepancies are probably due at least in part to the small counts and to differential viability; there may also have been differences in proportions of crossing over between the attached-X stock and those on which the calculated values are based.

A comparison can also be made between calculated and observed frequencies of offspring heterozygous for all the genes involved. The calculated value is 39.9 on the basis of table 4 (corrected) and 40.9 on the basis of table 8. The value observed by STURTEVANT (1931) is 34.5; and from the data given by BEADLE and EMERSON (1935, table 2) it appears that among 1478 offspring whose genetic constitutions were tested, 668 were heterozygous for all genes from scute to forked inclusive, this being a proportion of 45.2. The calculated values are almost precisely half way between the observed values. It should be noted also that STURTEVANT's figure is based on tests of only 383 wild-type daughters; and that the proportions of crossing over in the attached-X stock used by BEADLE and EMERSON were somewhat different from those of the stocks on which the calculated values are based.

The proportion of completely heterozygous individuals in attached X' is equal to $x_0+qx_1+q^2x_2+q^3x_3+ \cdots +q^nx_n$ provided that at any le of crossing over only two strands are involved; hence it is precisely equ to the proportion of non-crossovers in the same region among offsprin of females with unattached X's. The variations in the frequencies of x and p's compensate for one another, and the result is therefore ind pendent of the value of p. Nevertheless a figure obtained on the assum tion of crossing over between sister strands would not be significant despit its agreement with observation; for the tetrad frequencies on which i would be based are incorrect, involving as they do negative values. Th agreement would therefore be purely formal since it would be due to compensating errors.

The proportion of homozygosis for each gene can also be calculated. This is done by adding the proportions of the appropriate classes in the diagrams on page 182. If $p=1/2$, the sum is

$$\tfrac{1}{4}x_1+\tfrac{1}{8}x_2+\tfrac{3}{16}x_3+\tfrac{5}{32}x_4+ \cdots$$

where the x's are the frequencies of tetrads that are crossovers at 1, 2, 3, 4, · levels between the point of attachment and the gene in question. Values have been calculated on the basis of the $p=1/2$ columns of table 4 (corrected for crossing over between *f* and *bb*) and of table 8. These, together with observed values, are given in table 10 and illustrated in figure 4.

The solid curves in figure 4 illustrate homozygosis plotted against actual map distance in each cross. The two lines coincide almost completely from the spindle fibre to about *v*; beyond *v* the curve based on table 4 rises above that based on table 8.

The total map distance is almost exactly the same in the two crosses; but corresponding regions do not always have the same length. Hen while the ends of the curves lie in the same perpendicular, the other co sponding loci do not. In order to facilitate the comparison of correspond genes, the curves have been redrawn so that the abscissa of each gene the average of the values in the two crosses. The results are the das line for the data of table 4 and the dot-and-dash line for those of table 8

The theoretical and observed results are of the same general typ from nearly 0 at the proximal end to above 16 2/3 (the value exp pure chance) at the distal end. The greatest discrepancies are values observed by Emerson and Beadle in the region from *ca* these are undoubtedly due to the fact that there was less cross in the attached X stock used by Emerson and Beadle than in t of tables 4 and 8. Differences in crossing over and in coincidence account in part for the discrepancies in *ct* and more distal genes; but ential viability is suggested by the low value of *cv* as compared with *ct* and of *sc* as compared with *y* in some of the data.

The theoretical curve based on table 4 and on the *y bi cv ct v g B bb* cross was exhibited at the Sixth International Congress of Genetics and before the Genetics Society of America in 1932. Homozygosis when sister strands do not cross over was independently calculated by SAX (1932) and

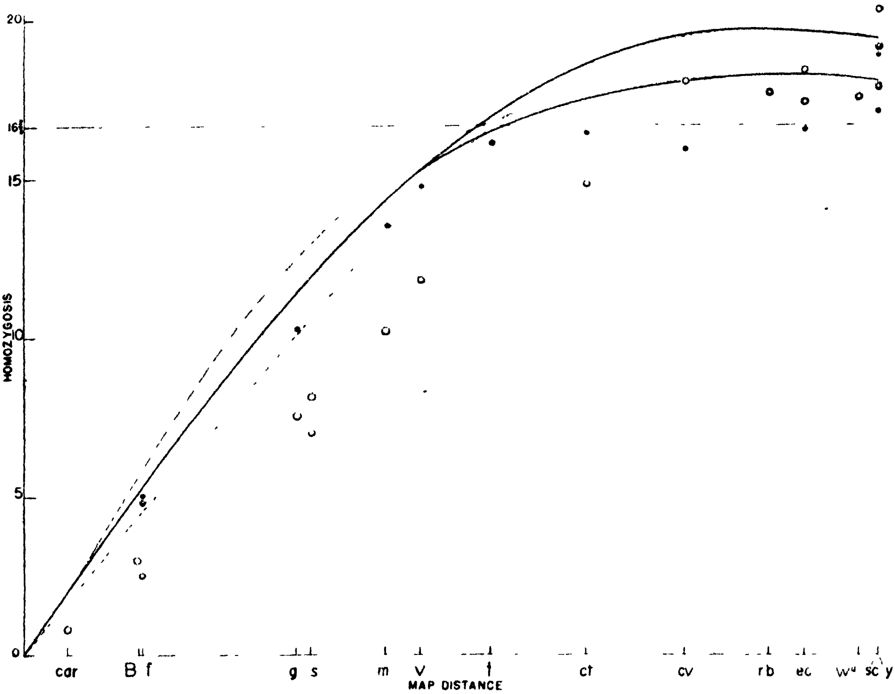

FIGURE 4. Proportions of homozygosis for recessive genes in offspring of heterozygous attached-X females. The abscissa indicates distance from the spindle fibre. The curves are drawn through the theoretical values given in table 10. The observed values in table 10 are also indicated; those from ANDERSON, L. V. MORGAN, and STURTEVANT by dots enclosed in circles; those from RHOADES by concentric circles; those from EMERSON and BEADLE by simple circles.

subsequently by BELLING (1933), KIKKAWA (1933)[2] and MATHER (1935); the data on which these calculations were based did not include the entire length of the X chromosome. BEADLE and EMERSON (1935) have obtained a similar curve from an analysis of crossing over in the attached X's themselves.

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[2] KIKKAWA's previous (1932) method was, as he himself pointed out, incorrect because the highest frequencies of homozygosis yielded by it were distinctly less than 16 2/3 percent. KIKKAWA in 1932 stated that the writer's theory as embodied in formula 2 of the present paper would not allow for *aaa/aba* individuals. This objection is answered by the analysis given above, which shows that such individuals are expected and with a frequency corresponding to the one observed experimentally. KIKKAWA's objection was due to a misinterpretation of the writer's theory; special cases of the theory have since been used by KIKKAWA himself.

The agreement of a theory with observation is of course not significant unless other theories agree less closely. It is therefore necessary to consider how the curve would be modified if the assumptions on which it was calculated were altered.

For regions near the point of attachment the curve is a straight line, the homozygosis being half the map distance from the spindle fibre. This would be expected on random association without sister strand crossing over; because homozygosis for a given gene is 1/4 of the frequency of tetrads of rank 1, while the map distance is 1/2, and there are few if any tetrads of higher rank. If sister strands cross over freely, homozygosis is 1/6 and map distance 1/3 of rank-1 tetrads; so that the agreement is equally good and this part of the curve cannot be used as evidence for or against sister-strand crossing over. But the distal part of the curve, as has been pointed out by the writer (WEINSTEIN 1932c) supplies the necessary evidence; for if sister strands crossed over, the proportion of homozygosis (as can be seen from the first diagram on page 182) would be

TABLE 10*

Percentages of homozygosis in daughters of attached-X females.

	CALCULATED		OBSERVED			
GENE	TABLE 4 $p = 1/2$ CORRECTED FOR CROSSING OVER BETWEEN *l* AND *bb*	TABLE 8 $p = 1/2$	ANDERSON 1925 L. V. MORGAN 1925 STURTEVANT 1931 SUMMARIZED BY STURTEVANT	RHOADES 1931	EMERSON AND BEADLE 1933	EMERSON AND BEADLE 1935
car		2.2				0.8
B			4.1		3.0	
f	4.6	5.9	5.1	4.9		2.5
g	10.1		10.3		7.5	
s		13.0			8.1	7.0
m			13.5		10.2	
v	14.6	15.8	14.8		11.9	11.8
lz			14.9			
l			16.1			
ct	18.4	17.6	16.4			14.8
cv	19.5	18.1	15.9			18.0
rb				17.6		
ec	19.6	18.3	16.6	17.3		18.4
w or w^a			16.5 (*w*)	17.5 (w^a)		
sc	19.4	18.0	17.1	17.9		20.3
y			19.0	19.2		

---

* This table includes all the data hitherto published. The proportions headed RHOADES (1931), and EMERSON and BEADLE (1933), have been calculated by the writer from the total counts of these workers The homozygosis values calculated by RHOADES differ slightly from those recorded here because he excluded some of his data.

In the experiment of EMERSON and BEADLE (1933) the value marked *B* is the average for Bar and its normal allelomorph.

$$\tfrac{1}{6}x_1+\tfrac{1}{6}x_2+\tfrac{1}{6}x_3+\cdot\cdot\cdot+\tfrac{1}{6}x_n$$

so that even if all tetrads were crossovers, the amount of homozygosis would not exceed one-sixth. The fact that both calculated and experimental curves rise above this level shows that crossing over does not occur freely between sister strands.

The curve also proves that only two of the four strands cross over at any level. For if all four always crossed over, no homozygosis would be produced at all; and if all four sometimes crossed over, the homozygosis in the proximal part of the curve would not be half the map distance but less. This is true regardless of whether crossing over occurs between sister strands.

The curve can also be used to test whether recurrence is random. That the proportion of homozygosis depends on the nature of recurrence can be seen from the fact that in tetrads of rank 2 homozygosis of genes distal to both exchanges results not from regressives or digressives but only from some of the progressives. If there is no crossing over between sister strands, homozygosis is produced only by those progressives in which the proximal crossing over is lateral (the distal crossing over must then be lateral also); this type is illustrated in figure 2B. The terms lateral and diagonal are applied according to the relative positions of the strands in the region of crossing over, and do not necessarily describe their relation at the proximal end.

If recurrence is random, these progressives constitute one-half of all progressives or one quarter of all rank-2 tetrads.[3] That is, the same proportion of rank-2 progressives as of single crossover tetrads produce homozygosis; while regressives, digressives, and non-crossovers do not produce it at all.

The substitutions indicated in equations 8–13 and table 7 can be tested with respect to their effect on homozygosis for scute with the aid of the following table.

TABLE 11

Tetrad frequencies for the X chromosome (random recurrence, no sister-strand crossing over)

RANK	0	1	2 REGRESSIVE	2 PROGRESSIVE	2 DIGRESSIVE	3	4
Table 4 (corrected to include crossing over between *f* and *bb*)	557	15678	2755	5510	2755	888	96
Table 8	904	7824	1730	3460	1730	488	

Equation 8. The replacement of non-crossovers and digressives by singles would increase homozygosis. In the corrected Xple figures, since

there are only 557 non-crossover tetrads there could be not more than 1114 additional singles, of which 278.5 would produce homozygosis for scute; this would raise the proportion from 19.4 to 20.4—not so good an agreement with the observed values for *sc* and *y*.

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[3] All these proportions must be divided by 2 when homozygosis for the recessive gene only is considered.

The reverse substitution (non-crossovers and digressives for singles) could have a much greater effect. If all of the 15678 singles were replaced, homozygosis for *scute* would fall to 5.5, an impossibly low figure. A reduction of homozygosis to 16.7 would require the replacement of 1/5 of the singles; this is highly improbable, since most of the observed values for *sc* and *y* differ significantly from 16.7.

On the basis of table 8, the highest value would be 20.8, the lowest 5.9; and a decrease to 16.7 would involve replacing 1/9 of the singles.

Equation 9. The progressives could be doubled or eliminated; and homozygosis would fall as low as 14.6 or rise as high as 24.2 on the basis of table 4 (corrected). The corresponding values on the basis of table 8 would be 12.6 and 23.4. Hence only a small part of the substitutions would be possible.

Equation 10. This would not alter homozygosis, since the effectiveness of a progressive is equivalent to that of a single.

Equation 11. Homozygosis would remain unaltered because a non-progressive tetrad of rank 3 has the same effect as a single. This follows from the fact that in the rank-3 tetrad two of the crossings over are regressive with respect to each other and neutralize each other; the third crossing over effects homozygosis if it is diagonal but not if it is lateral.

If sister strands cross over freely, homozygosis results from 1/3 of the tetrads of rank 1 and 1/2 of the progressives of rank 2, but not from regressives or digressives of rank 2 or from non-crossovers (see footnote 3). Since homozygosis calculated on free sister-strand crossing over and random recurrence is too low, only those deviations from random recurrence are possible that raise the calculated proportion to the observed level.

The method by which the negative frequency of non-crossovers was eliminated (table 7) consisted in decreasing the number of tetrads of rank 1 by 10632 and increasing the digressives by 5316. This would decrease the proportion of homozygosis for distal genes and is therefore impossible.

Of the substitutions indicated in equations 8, 11, 12, and 13 the only ones that would increase homozygosis are the replacement of the left-hand by the right-hand side of equations 8 and 12. But singles cannot be increased by the substitutions indicated in equation 8, since there are no non-crossovers at whose expense this could be accomplished; hence the only substitution that remains is of progressives for regressives and digressives (equation 12). To test this completely the figures in table 7 do not suffice, since they include only the region from *forked* to *scute;* but they can be considered as roughly applicable to the region from the point of attachment to *crossveinless* or *echinus*, which is about as long as that from *forked* to *scute.* On this basis there might be as many as 10820 additional progressives; and the proportion of homozygosis due to singles and progressives might be increased to as much as 1/28239 (1/6 4698+1/4 22016) = 22.2—too high a figure, so that only part of the substitution is possible.

POLYPLOIDS

Triploids

In triploids there is evidence that only two of the six strands cross over at any level (BRIDGES and ANDERSON 1925, REDFIELD 1930). Hence among the strands emerging from a hexad which is a crossover at a given level, the proportion that are recognizable crossovers at that level is the product of 1/3 by the chance of recognizing a crossover strand once it is recovered. This chance depends on two factors: (1) whether all three chromosomes are distinguishable from each other or two are indistinguishable, and (2) whether crossing over takes place between sister strands. The results for four possible cases are as follows:

	CROSSING OVER BETWEEN SISTER STRANDS AS FREQUENT AS ON CHANCE	NO CROSSING OVER BETWEEN SISTER STRANDS
All 3 chromosomes distinguishable	1/3(4/5) = 4/15	1/3
2 chromosomes indistinguishable	1/3(8/15) = 8/45	1/3(8/12) = 2/9

These fractions represent the chances of detecting a crossing over at one level if one chromatid is recovered ("regular" offspring). If two chromatids are recovered ("exceptional" offspring), then p for hexads is the product of the chance that *at least* one is a crossover by the chance of recognizing a crossover strand once it is obtained provided that the two chromatids are recovered at random.

BRIDGES and ANDERSON (1925) showed that a chromosome may cross over with different chromosomes at different levels; and they concluded that synapsis involves all three chromosomes equally throughout their length. On this theory recurrence would be random for all six chromatids, except that sister-strand crossing over may be excluded. But when hexad frequencies are calculated on this basis, negative values are encountered (WEINSTEIN 1932c).

In table 12 are given the hexad frequencies calculated for the cross of REDFIELD (1930, table 1, broods 1 and 2 combined), which covers about half of the third chromosome. Here all three chromosomes are marked;

hence formula 2 can be applied with $p = 4/15$ and $p = 1/3$. When $p = 4/15$, the largest negative value (-135) is 1.6 times its standard error. When $p = 1/3$, the negative value (-30) is 0.6 times its standard error. The results are not conclusive; still they suggest that with longer stretches of chromosome significant negative frequencies would appear.

TABLE 12

Crossing over in triploids of constitution
$$\begin{array}{ccccc} + & D & ma & Sb & + \\ \hline se & Mh & + & + & H \\ \hline h & + & cu & bx & e^s \end{array}$$

REGIONS OF CROSSING OVER	OBSERVED FREQUENCIES REDFIELD 1930 n = 1030	TETRAD FREQUENCIES CALCULATED FOR RANDOM RECURRENCE $p=1/2$	$p=1/3$	$p=4/15$
0	565	272	127	101
1	87	100	63	17
2	171	226	183	97
3	70	56	−30	−135
4	47	48	21	−7
12	22	76	144	193
13	11	36	63	77
14	8	20	18	−4
23	27	104	225	341
24	12	40	72	91
34	6	20	36	46
123	1	8	27	53
124	2	16	54	105
134	1	8	27	53

Negative values are in fact obtained for the cross covering almost the entire length of the third chromosome given in REDFIELD's table 5. Here only one of the chromosomes is marked, hence $p = 2/9$. And a further modification of procedure is necessitated by the fact that since there are two strands of one kind and one of the other, the chances of recognizing crossing over at different levels are not independent, even though recurrence be random. It becomes necessary therefore to use equations like those in case 3. The analysis need not be given in detail because a similar one has been made by other workers (MATHER, 1933, 1935; KIKKAWA, 1934; see KIKKAWA for the equations). These negative frequencies are not in themselves significant; but they point in the same direction as those in the previous cross.

These results suggest that, for considerable distances, only two of the chromosomes conjugate while the third goes unmated to either pole. On this assumption, the unmated chromosome region can be neglected; for the conjugating chromosomes form what is essentially a tetrad, and the chromosome recovered in regular offspring is always one of those that conjugate. Hence the data in table 12 can be treated as in diploids; that is, formula 2 can be applied with $p=1/3$ if sister strands cross over and $p=1/2$ if they do not. When $p=1/3$, as we have seen, some negative values are encountered, though their significance is doubtful; when $p=1/2$, however, all frequencies are positive and support the theory on which they are based.

If the conclusions suggested by these results are correct, we should expect that when two strands are recovered (exceptional offspring), usually not more than one strand will be a crossover. This is borne out by the results with the X chromosome: from BRIDGES and ANDERSON (1925 table 5) it appears that a crossover chromatid is associated with a non-crossover 82 times out of 97, and with a crossover only 15 times. The tendency is complete at the spindle fibre and decreases for more distal regions because of crossing over between them and the spindle fibre. In the autosomes there is a similar relation between association of chromosomes and distance from the spindle fibre (REDFIELD 1930). As has been pointed out by MULLER (unpublished, cited by WEINSTEIN 1932c), these facts support the view that only two of the chromosomes conjugate while the third remains unmated, although there may be some change of partners.

Similar conclusions concerning conjugation in triploids have been reached by MATHER (1933, 1935); and on cytological grounds by BELLING (1921), MULLER (1922) and DARLINGTON (1932). RHOADES (1933) and KIKKAWA (1934) have presented somewhat different interpretations. The questions raised by these workers cannot be discussed here but will be treated elsewhere.

Frequency of crossing over in diploids and triploids

In comparing the amount of crossing over in diploids and triploids, it is necessary to distinguish between crossing over per strand and crossing over per tetrad or hexad (WEINSTEIN 1932a).

If in any given region crossing over is constant per chromatid, then if chromatids are recovered at random there would be no change in crossing over per chromatid in triploids if sister strands do not cross over, but an increase if they do. For in the latter case one third of the crossings over in a tetrad would be undetectable, whereas in the hexad the proportion would be only $3/15=1/5$. This was pointed out by BRIDGES and ANDERSON (1925), who suggested that thus it might be possible to discover whether or not crossing over occurs between sister strands.

There is however another possibility which they did not consider; namely, that the amount of crossing over might be constant not per strand but per group of chromatids (tetrad or hexad). In this case the amount per chromatid would be decreased, since there would be the same number of crossings over but more strands. The ratio of recognizable cross-over strands in triploids to that in diploids would then be $4/15 \div 1/3 = 4/5$ if sister strands cross over, and $1/3 \div 1/2 = 2/3$ if they do not; provided of course that strands are recovered at random. Here again it might be possible to discover whether sister strands cross over.

In BRIDGES and ANDERSON's data for the X chromosome, the amount of crossing over per strand recovered was half as great in triploids as in diploids (except for the leftmost region of the X, which showed an increase). If strands were recovered at random, this would agree better with the supposition that crossing over is the same for hexad as for tetrad and that sister strands do not cross over. But the decrease is too great and suggests that non-crossover strands are recovered more often than on chance. If two strands are recovered and one is usually a non-crossover, the amount of crossing over per recovered strand in triploids would be approximately halved, as it is; hence the figures support the theory that only two strands usually associate in synapsis while the third goes unmated to one pole. We may still conclude that crossing over per hexad is the same as per tetrad; but no conclusion can be drawn directly as to crossing over between sister strands, for the result would be the same whether this occurs or not, since the hexad acts essentially as a tetrad.

At the left end of the X, where there must be a greater tendency for strands to be recovered at random (because of crossing over between this region and the spindle fibre), the ratio might be expected to approach 2/3 if sister strands do not cross over; instead it rises to 2. This increase remains unexplained.

In REDFIELD's data, since only those individuals were selected that did not receive the unmated chromosome, the triploid-diploid ratio per strand might be expected to decrease from 1 at the spindle fibre to about 2/3 for distal regions, where strands are recovered more nearly at random. The latter figure is realized near the ends of the chromosome; and there is a rise toward the center. Near the spindle fibre however the ratio increases to 3 or 4; and this increase is not accounted for.

MULLER (unpublished, see WEINSTEIN 1932c) and RHOADES (1933) have reached similar conclusions, though RHOADES's interpretation of synapsis in triploids differs from that given above.

Higher polyploids

The multiple-strand theory can be applied to higher polyploids; it is merely necessary to take into account which chromosomes are distinguishable and how they undergo synapsis. This may result in complicated situations; but where (as in some tetraploids) synapsis is in pairs, the treatment is not essentially different from that of diploids.

SUMMARY, WITH SOME CONSIDERATION OF THE MECHANISM OF CROSSING OVER

A mathematical method is described which makes it possible to calculate from the observed frequencies in a crossover experiment (1) the frequency of undetected crossing over including that between sister strands, which cannot be recognized directly, and (2) how the individual chromatids are associated in tetrads. The method can be applied to ordinary diploids, to cases of non-disjunction and attached chromosomes, and to polyploids. The calculated results differ according to the assumptions made as to crossing over at any given level and the mutual relations of crossings over at different levels; and since some of the results are inconsistent with the experimental data or (as in the case of negative frequencies) meaningless, the assumptions on which they rest can be ruled out and our knowledge of the mechanism of crossing over thus becomes more precise.

The experimental results when subjected to this mathematical treatment lead to the following conclusions regarding the mechanism of crossing over:

(1) There is no crossing over between sister chromatids.

(2) At any level only two of the four chromatids may cross over.

(3) Otherwise it is a matter of chance which chromatids cross over at any level.

(4) The chromatids that cross over at one level do not determine which ones cross over at other levels.

(5) This mechanism implies that for inclusive coincidence the true value for strands or tetrads is identical with the observed value regardless of whether sister strands cross over or not; for select coincidence the tetrad value is less than the observed value where the two differ; and for partial coincidence the tetrad and the observed values are in general not identical, though they may be very similar. If recurrence is not random, all types of tetrad coincidence will in general differ from the observed values.

Coincidence for chromatids remains identical with the observed values if sister strands do not cross over.

All these propositions and others are summed up in formula 2 for $p = 1/2$; that is, that the frequency of any class of tetrads is given by

$$X = 2^r[a_0 - a_1 + a_2 - a_3 + \cdots + (-1)^n a_n].$$

(6) In triploids the same mechanism holds; but the evidence indicates

that two of the chromosomes undergo synapsis to the exclusion of the third, over considerable distances, though there is some change of partners; but recurrence is probably random among the strands of the conjugating chromosomes.

Deviations from the mechanism described above lead to results that are inconsistent or incorrect:

(1) Crossing over between sister chromatids would involve negative frequencies of tetrads, would not allow the proportion of homozygosis for any gene to exceed 16 2/3 percent in cases of attached X's and non-disjunction and would result in other discrepancies. Evidence in the same direction is available in the work of EMERSON and BEADLE (1933) and BEADLE and EMERSON (1935) on attached X's; and of L. V. MORGAN (1933) on the ring-shaped X; and in the non-occurrence of sister-strand crossing over in Bar either in its ordinary locus (STURTEVANT 1925) or when translocated to the left end of the chromosome (MULLER and WEINSTEIN 1932 and unpublished data).

(2) Crossing over between more than two chromatids at a given level would diminish or eliminate homozygosis in offspring of attached X's. This conclusion is supported by the non-occurrence of identical crossovers in offspring of attached X's (ANDERSON 1925b) and of triploids (BRIDGES and ANDERSON 1925).

(3) Random occurrence of crossing over is shown most simply in the equality of lateral and diagonal crossing over near the spindle fibre (or a 2:1 ratio if sister strands cross over); and this involves random occurrence in other regions to give the observed results.

(4) Deviations from random recurrence would, if too great, lead to negative frequencies, and would modify the proportions of homozygosis in attached X's from the observed values.

The above results would follow the modification of one condition at a time. Modification of two or more conditions simultaneously might increase discrepancies; for example, the crossing over of all four chromatids at the same level would produce some of the same effects as an increase of digressive crossing over. On the other hand, some modifications would compensate for one another: an increase in digressive crossing over will counteract some effects of increased regressive crossing over, and it may also eliminate negative frequencies produced by crossing over between sister strands.

The conclusions have been stated in terms implying that at the time when crossing over occurs each chromosome is split into two separate strands or rows of genes. This assumption is not required by the mathematical analysis, and it leads to some difficulties which, together with the modifications they suggest, will be briefly considered.

(1) Crossing over between diagonal chromatids might prevent interchange between two others at the same or neighboring levels by keeping them apart; but it is not obvious why a lateral crossing over should have the same effect. This suggests that when crossing over occurs sister chromatids are not completely independent of each other.

(2) It is difficult to see why two completely independent sister chromatids should not cross over with each other, particularly since a sister chromatid might change positions with a homologous one by crossing over with it. The evidence however does not actually show that sister strands do not cross over: such crossings over are not directly detectable since they result in no recombination of characters. Our criterion of their occurrence is really whether crossing over between homologous strands at the same or neighboring levels is prevented, and the evidence shows that it is not. This could be explained if the sister genes are not arranged in two distinct rows but the genes at one level are oriented at random with respect to the genes at other levels; or to put it somewhat differently, there would be one row of double genes instead of two rows of single genes, and there would be no sister strand crossing over because there would be no sister strands (WEINSTEIN 1932c).

It is not necessary however to assume that orientation of genes at one level is random with respect to all other levels; but only that it is random with respect to the next level at which crossing over occurs— a considerable distance, being in the X chromosome of Drosophila some 14 units or about one-fifth of its genetic length. It would be sufficient if within this distance orientation of genes were random on two sides of one interlocus; or if genes did tend to form two strings, but the tendency of two genes to remain in the same string decreased with distance between them and finally disappeared when the distance became long enough for a second crossing over to occur. This might help to explain also why there is crossing over at all.

(3) It is difficult to understand why two chromatids that are sufficiently closely associated to exchange parts are no more likely to associate together at the next crossing over than either is to associate with a third. If at levels sufficiently far apart the orientation of sister genes is random, this difficulty disappears.

Thus all three difficulties suggest that the two chromatids of a chromosome are not entirely separate strands, and two of the difficulties can be explained if the orientation of sister genes at one level is random with respect to the sister genes of the same chromosome at other levels at which crossing over occurs.

The limitation of crossing over at a given level to two chromatids requires however further explanation. If both sister genes are already formed at the time that crossing over occurs, it must for some reason be difficult or impossible for both to become detached from their neighbors in the chromosome. This might be due to the persistence of a material connection between successive genes, or of some physical force.

It is possible however that both sister genes are not already formed before crossing over takes place; particularly if the new genes are formed not by division of the old ones but by being catalyzed by them. The limitation of crossing over could then be explained if the old genes remain linked at the points of crossing over and only the new genes can become attached to new genes of the other chromosome, more or less as BELLING (1933) postulated. This might be brought about by the formation of the new genes on different sides of the old genes at different levels. There would still however have to be emerging chromatids made up of old and new genes of the same chromosome, to account for progressive crossing over; and this would come about if as the chromatids separate, old and new genes move at random at different levels of crossing over, as has been suggested above.

HISTORICAL NOTE

The part of the present investigation dealing with random recurrence and constant p was carried out in 1928. An abstract published in that year (WEINSTEIN 1928) included a statement of the problem, the general formula numbered 2, the invariance of coincidence under certain conditions, and the applicability of the method to attached X's and polyploids. The conclusion was drawn that association of strands in crossing over can not be entirely a matter of chance; this was based on the application of the formula for various values of p, including 1/3 (free sister-strand crossing over) and 1/2 (no sister-strand crossing over). The crossover frequencies worked out by BELLING (1931) and SAX (1932) correspond to the case $p = 1/2$ if different classes of the same rank are not separated.

The theory was generalized by WEINSTEIN (1930); and the complete theory was presented in a paper and exhibits at the Sixth International Congress of Genetics (WEINSTEIN 1932a, 1932b). The exhibits included the originals of all the figures in the present paper, but the additional data based on table 8 have since been added to figure 4. The work on attached X's and triploids was reported in the same year (WEINSTEIN 1932c).

The derivation of formulas by sets of equations, as given in the present paper, was included in the writer's report before the Congress of Genetics in 1932. Special cases of such sets of equations have since been used by MATHER (1933, 1935) and KIKKAWA (1934).

I wish to thank Miss Grace E. Jones of the University of Minnesota for drawing figure 3; and Mr. A. O. Babendreier of the Johns Hopkins University School of Engineering for drawing figures 1, 2, and 4.

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THE TRISOMIC DERIVATIVES OF OENOTHERA LAMARCKIANA

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INTRODUCTION

IN DESCRIBING the chromosome configurations in certain trisomic oenotheras, CATCHESIDE (1933) has shown how the origin and characteristic behavior of the trisomic forms can be accounted for by the translocation interpretation of multivalent ring formation.[1] There are, however, so many respects in which the trisomic derivatives of the oenotheras differ from those of other organisms that a fuller discussion of the entire problem is desirable. Since the derivatives of *Oenothera Lamarckiana* have been studied in greater detail than those of other species, the following discussion will be confined almost entirely to the former, though the situations described should be very much the same in other species of the genus in which there are large chromosome rings.

The principal respects in which the trisomics of *Oe. Lamarckiana* differ from similar forms in other genera are: 1, the higher frequencies in which the trisomic forms appear in the progenies of diploids; 2, the larger number of distinct trisomic types produced directly from the diploid; 3, the larger number of "secondary" trisomic types produced in the progenies of the "primaries"; 4, the production of identical types both as "primaries," directly from the diploid, and as "secondaries," in the progenies of other trisomics; 5, the dissimilar breeding behavior observed in different trisomics: whereas certain trisomic derivatives of *Oe. Lamarckiana* always throw the ancestral, diploid type in their progenies, as do the trisomics of most other organisms, other trisomic derivatives of *Oe. Lamarckiana* breed true for the trisomic condition.

By the application of the translocation interpretation to the observed cytological behavior of the diploid and trisomic forms, these peculiarities of the oenothera trisomics may be readily accounted for. The following sections of the present discussion will treat each of these peculiarities separately in their relation to the currently accepted interpretations.

CHROMOSOME DISJUNCTION IN HETEROZYGOUS TRANSLOCATIONS

In forms heterozygous for a reciprocal translocation, the four chromosomes which make up the closed ring may separate in any of three ways in the first meiotic anaphase. Only one of these types of disjunction gives products carrying the full complement of chromosome ends, and this is the type illustrated in figure 1A in which the chromosomes assume a zigzag orientation on the meiotic spindle so that each chromosome separates from the two adjacent chromosomes which are homologous to it over parts of their lengths. In the other two types of disjunction (figures 1B and 1C), adjacent chromosomes pass to the same pole, and the products in each instance are deficient for one chromosome arm and carry another arm in duplicate.

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[1] Earlier, CLELAND (1929b) had discussed the relation of non-disjunction of ring-chromosomes to the production of trisomics, but this was not done in relation to the translocation interpretation and consequently certain erroneous conclusions were drawn.

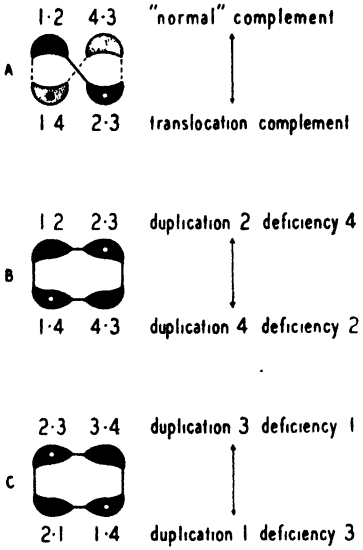
1·2 4·3
"normal" complement
A
1 4 2·3
translocation complement
1 2 2·3
duplication 2 deficiency 4
B
1·4 4·3
duplication 4 deficiency 2
2·3 3·4
duplication 3 deficiency 1
C
2·1 1·4
duplication 1 deficiency 3

FIGURE 1.—Diagrams of the three types of disjunction possible in a ring of four chromosome The arrows indicate the directions in which the chromosomes separate.

In *Drosophila melanogaster*, gametes carrying a net duplication for one chromosome arm and a deficiency for another are functional provided they meet gametes carrying the complementary product of the same type of disjunction (B or C figure 1). Genetic data indicate that the regular, zigzag disjunction occurs in approximately 60 percent of the instances in one such translocation (DOBZHANSKY and STURTEVANT 1931), and one or the other of the two non-disjunctional types in the remaining instances.

In plants, on the other hand, microspores receiving a deficiency for one chromosome arm and a duplication for another do not develop into functioning pollen. *Zea mays* heterozygous for a reciprocal translocation produces, in most instances, about 50 percent of small, empty pollen grains, and the regular, zigzag disjunction has been observed to occur in about half of the microspore mother-cells and the non-disjunctional types in the other half (BRINK and BURNHAM 1929, BURNHAM 1930, MCCLINTOCK 1930, BRINK and COOPER 1932).

In oenotheras heterozygous for a single reciprocal translocation, the ring chromosomes almost invariably separate in the regular, zigzag manner. Such an oenothera is the hybrid *flavens stringens* in which CLELAND and OEHLKERS (1930) found but two instances of non-disjunction in a total of 109 sporocytes examined. Consequently there is little or no bad pollen produced by oenotheras with small rings of chromosomes; for example *velans*·^{h}*Hookeri*, DAVIS' strain of *Oe. franciscana*, etc. In oenotheras heterozygous for a large number of translocations, that is in oenotheras with large rings of chromosomes, the non-disjunctional types occur more frequently. These types of disjunction will be described for *Oe. Lamarckiana*.

CHROMOSOMAL CONSTITUTION OF OE. LAMARCKIANA

Oenothera Lamarckiana is a heterogametic species in which one haploid set of chromosomes is not identical with the other. Of the seven chromosomes making up each set, only one is common to both; the remaining six chromosomes of one set represent translocations of the six remaining chromosomes of the other set. Hence, in the ordinary diploid form of this species there are thirteen chromosomes of more or less different homologies, only one of which is represented in duplicate.

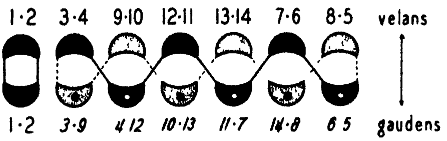

FIGURE 2.—Diagram of the chromosome configuration at metaphase or early anaphase in *Oe. Lamarckiana*.

Note—In this and succeeding diagrams the following conventions are used the ring-chromosomes belonging to the *gaudens* complex are distinguished by dots within the chromosomes and by sloping numerals (in the text these chromosomes are represented by Italics, the *velans* ring-chromosomes and the pairing chromosomes by Roman type); whenever the chromosomes form a closed ring, as in this diagram, the ring is represented in side view with the chromosomes in the foreground drawn in solid black and those in the background lightly shaded.

The two haploid sets of chromosomes in *Oe. Lamarckiana* are known respectively as the *velans* and *gaudens* complexes. All the chromosomes making up the *velans* complex have been identified (CLELAND and BLAKESLEE 1930, EMERSON and STURTEVANT 1931, RENNER 1933). The chromosomes of *velans* are designated 1·2, 3·4, 5·8, 6·7, 9·10, 11·12, 13·14, in which each number represents a particular chromosome end. The chromosomes of the *gaudens* complex have not been completely identified. Chromosomes 1·2 and *5·6* are known to be present in this complex, and one of the remaining possibilities for the other chromosomes is *3·9*, *4·12* *7·11*, *8·14*, *10·13*, which will be used tentatively in this treatment for which an exact identification is not essential.

The pairing of homologous regions of the chromosomes of the two complexes brings twelve of the chromosomes into a large ring in which the chromosomes of *velans* and *gaudens* must alternate as indicated in the accompanying diagram (figure 2). Then the regular, zigzag disjunction of chromosomes in anaphase must separate the *velans* ring-chromosomes from those of *gaudens* and only the two normal complexes should result.

IRREGULAR DISJUNCTION OF CHROMOSOMES

While, as a rule, adjacent ring-chromosomes pass to opposite poles in meiosis, it is often observed, especially in the case of large rings, that adjacent chromosomes may pass together to the same pole. CLELAND (1929a) examined 358 microsporocytes of *Oe. Lamarckiana* in each of which the ring of twelve chromosomes was intact Of these 67, or 18.7 percent, had irregularities in the zigzag arrangement of chromosomes.

In a ring made up of an even number of chromosomes such irregularities cannot occur singly, since if two adjacent chromosomes pass to one pole it is impossible for all remaining chromosomes to assume the zigzag orientation. The two sets of irregularities which must occur may have different relative positions in the ring, and the products resulting will differ depending upon the positions of the irregularities.

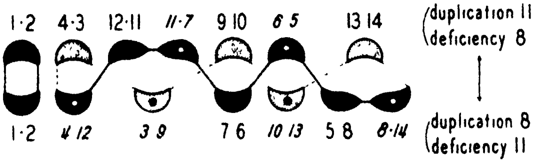

FIGURE 3. - Non-disjunction of ring-chromosomes in *Oe Lamarckiana* from which seven chromosomes are recovered in each product

Numerically-equal non-disjunction

In those instances in which one set of irregularly disjoining chromosomes passes to one pole and the other set to the other pole (see CLELAND 1929a, figure 23) the disjunction of chromosomes is numerically equal. In this sort of separation, however, the two complexes are not recovered intact, but at each pole there is a mixture of *velans* and *gaudens* chromosomes. At one time (CLELAND 1929b) it was thought that such irregularities might be the basis of genetic crossing over in these forms. Since the establishment of the translocation hypothesis of ring formation, however, it is known that such irregularities in disjunction must give rise to inviable products which will contribute largely to the bad pollen and inviable eggs observed in *Oe. Lamarckiana*. The distribution of homologous segments of chromosomes in disjunctions of this sort is illustrated in figure 3. One daughter cell receives one chromosome arm in duplicate, for example arm 11, but lacks another arm entirely, arm 8 in this instance, whereas the other daughter nucleus fails to receive the former and has the latter in duplicate. Spores in which there is a deficiency for one chromosome arm and a duplication for another generally fail to produce functioning pollen, as has been well illustrated in the different examples of translocations in Zea (BRINK and BURNHAM 1929, BURNHAM 1930, BRINK and COOPER 1932, etc.).

Numerically unequal disjunction

In other instances (CLELAND 1929a, figures 25, 26, CLELAND and OEHLKERS 1930, figures 1D, 4D, 6H, 9E, 11F, 12E, 12H, etc.), both sets of irregularly disjoining chromosomes pass to the same pole, resulting in an unequal distribution in which six chromosomes pass to one pole and eight to the other. Examples of this sort are illustrated in figures 4 to 6. The daughter cell receiving six chromosomes is deficient for two chromosome arms (11 and 14 figure 6) and should fail to develop either as megaspore or microspore. The cell receiving eight chromosomes, however, has a full complement of chromosome arms and, in addition, two arms in duplicate (ends 11 and 14 in the example illustrated). Eight-chromosome complexes of this sort generally fail to function as pollen but are readily transmitted through the eggs.

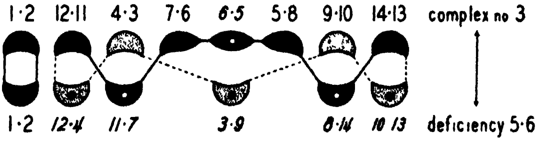

FIGURE 4.—Non-disjunction in which three adjacent ring-chromosomes pass to the same pole, resulting in an 8 to 6 distribution of chromosomes.

CLELAND (1929a and CLELAND and OEHLKERS 1930) has studied the frequency in which such numerically unequal divisions occur in different strains of *Oe. Lamarckiana.* The observed frequency was about 10 percent in the "1912 selfed" strain of SHULL, the *Lamarckiana* of DE VRIES and in the r-*Lamarckiana* of RENNER, but approximately 20 percent in the "1910 selfed" strain of SHULL and in *Oe. Lamarckiana cruciata.*

In the 8-chromosome products of such irregular disjunctions there are mixtures of *velans* and *gaudens* chromosomes; the extent of the mixing depends upon the relative positions in the ring of the two sets of irregularly disjoining chromosomes. In certain instances, such as illustrated by CLELAND and OEHLKERS 1930, figure 12E, three adjacent chromosomes may pass to one pole, in which case the cell receiving eight chromosomes has either a complete set of *velans* chromosomes together with one from *gaudens* (figure 4), or a complete set of *gaudens* chromosomes and one from *velans*, depending upon the exact positions of the irregularities. Since there are twelve positions in the ring at which such irregularities may occur, there will be twelve different 8-chromosome complexes (those numbered 1 to 12 in table 1) arising from this particular sort of disjunction.

Following a second type of unequal distribution, in which the two sets of adjacent chromosomes which pass to the same pole are separated by one chromosome which goes to the opposite pole (figure 5), the 8-chromosome products will again have seven ring-chromosomes, but there will be either five *velans* and two *gaudens*, or two *velans* and five *gaudens*. In each of the twelve different 8-chromosome complexes (13 to 24 in table 1) which arise from this particular type of disjunction, there is no one particular chromosome which can be considered as an extra chromosome; instead, such complexes carry net duplications for two chromosome arms which taken together do not represent any chromosome normally present in *Lamarckiana*. The same is true of the twelve complexes (25 to 36 in table 1) arising in the manner illustrated in figure 6. In such cases there are three regularly disjoining chromosomes lying between the two sets of non-disjoining chromosomes, and the 8-chromosome products have either four *velans* ring-chromosomes and three *gaudens*, or three *velans* and four *gaudens*, again depending upon the exact positions of the irregularities.

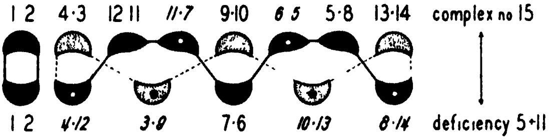

FIGURE 5.- Non-disjunction in which the 8-chromosome products have 7 ring chromosomes, including either 5 *velans* and 2 *gaudens* or 2 *velans* and 5 *gaudens*.

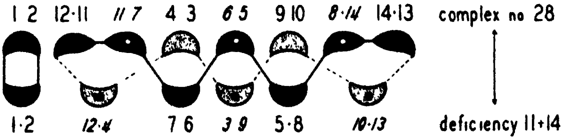

FIGURE 6 —Non-disjunction with 3 regularly disjoining chromosomes lying between the 2 sets of non-disjoining chromosomes, giving 8-chromosome products with 4 *velans* and 3 *gaudens* ring-chromosomes.

There is thus a total of thirty-six complexes with extra chromosomes which can arise directly from *Oe. Lamarckiana* as a result of irregularities in the distribution of the ring chromosomes. All these are composed of mixtures of *velans* and *gaudens* chromosomes in varying degrees. In addition, each represents a net duplication for two chromosome arms, but, as

TABLE 1

The thirty-six different 8-chromosome complexes produced directly from Oe. Lamarckiana by irregular chromosome disjunction.

1	1·2, 3·4, 3·9, 5·8, 6·7, 9·10, 11·12, 13·14	*velans*+ 3·9
2	1·2, 3·4, 4·12, 5·8, 6·7, 9·10, 11·12, 13·14	*velans*+ 4·12
3	1·2, 3·4, 5·6, 5·8, 6·7, 9·10, 11·12, 13·14	*velans*+ 5·6
4	1·2, 3·4, 5·8, 6·7, 7·11, 9·10, 11·12, 13·14	*velans*+ 7·11
5	1·2, 3·4, 5·8, 6·7, 8·14, 9·10, 11·12, 13·14	*velans*+ 8·14
6	1·2, 3·4, 5·8, 6·7, 9·10, 10·13, 11·12, 13·14	*velans*+10·13
7	1·2, 3·4, 3·9, 4·12, 5·6, 7·11, 8·14, 10·13	*gaudens*+ 3·4
8	1·2, 3·9, 4·12, 5·6, 5·8, 7·11, 8·14, 10·13	*gaudens*+ 5·8
9	1·2, 3·9, 4·12, 5·6, 6·7, 7·11, 8·14, 10·13	*gaudens*+ 6·7
10	1·2, 3·9, 4·12, 5·6, 7·11, 8·14, 9·10, 10·13	*gaudens*+ 9·10
11	1·2, 3·9, 4·12, 5·6, 7·11, 8·14, 10·13, 11·12	*gaudens*+11·12
12	1·2, 3·9, 4·12, 5·6, 7·11, 8·14, 10·13, 13·14	*gaudens*+13·14
13	1·2, 3·4, 3·9, 5·8, 6·7, 10·13, 11·12, 13·14	duplication 3+13
14	1·2, 3·4, 4·12, 5·8, 6·7, 7·11, 9·10, 13·14	duplication 4+7
15	1·2, 3·4, 5·6, 5·8, 7·11, 9·10, 11·12, 13·14	duplication 5+11
16	1·2, 3·4, 5·6, 6·7, 8·14, 9·10, 11·12, 13·14	duplication 6+14
17	1·2, 3·4, 5·8, 6·7, 8·14, 9·10, 10·13, 11·12	duplication 8+10
18	1·2, 3·9, 4·12, 5·8, 6·7, 9·10, 11·12, 13·14	duplication 9+12
19	1·2, 3·4, 3·9, 5·6, 7·11, 8·14, 10·13, 11·12	duplication 3+11
20	1·2, 3·4, 4·12, 5·6, 7·11, 8·14, 9·10, 10·13	duplication 4+10
21	1·2, 3·9, 4·12, 5·6, 5·8, 7·11, 10·13, 13·14	duplication 5+13
22	1·2, 3·9, 4·12, 5·6, 6·7, 8·14, 10·13, 11·12	duplication 6+12
22	1·2, 3·9, 4·12, 5·6, 7·11, 8·14, 9·10, 13·14	duplication 9+14
24	1·2, 3·9, 4·12, 5·8, 6·7, 7·11, 8·14, 10·13	duplication 7+8
25	1·2, 3·4, 3·9, 5·8, 6·7, 8·14, 10·13, 11·12	duplication 3+8
26	1·2, 3·4, 4·12, 5·6, 5·8, 7·11, 9·10, 13·14	duplication 4+5
27	1·2, 3·4, 5·6, 6·7, 8·14, 9·10, 10·13, 11·12	duplication 6+10
28	1·2, 3·4, 5·6, 7·11, 8·14, 9·10, 11·12, 13·14	duplication 11+14
29	1·2, 3·9, 4·12, 5·8, 6·7, 7·11, 9·10, 13·14	duplication 7+9
30	1·2, 3·9, 4·12, 5·8, 6·7, 10·13, 11·12, 13·14	duplication 12+13
31	1·2, 3·4, 3·9, 5·6, 6·7, 8·14, 10·13, 11·12	duplication 3+6
32	1·2, 3·4, 4·12, 5·6, 7·11, 8·14, 9·10, 13·14	duplication 4+14
33	1·2, 3·4, 5·6, 7·11, 8·14, 9·10, 10·13, 11·12	duplication 10+11
34	1·2, 3·9, 4·12, 5·6, 5·8, 7·11, 9·10, 13·14	duplication 5+9
35	1·2, 3·9, 4·12, 5·8, 6·7, 7·11, 10·13, 13·14	duplication 7+13
36	1·2, 3·9, 4·12, 5·8, 6·7, 8·14, 10·13, 11·12	duplication 8+12

can be seen from table 1, no single whole chromosome is represented twice in any one of these 8-chromosome complexes. Each of the first twelve complexes listed has a complete set of the chromosomes of one of the typical complexes (either *velans* or *gaudens*) and one chromosome of the other complex in addition. In these the net duplication corresponds to one of the chromosomes normally present in the diploid form. In the remaining twenty-four extra-chromosome complexes, however, the net duplication is in each instance for two arms which, taken together, do not correspond to any chromosome of either complex of the parent form. Phenotypically the first twelve may be considered as primary "trisomic" complexes and the remaining twenty-four as tertiary in that the net duplications represent possible new translocations. Actually, however, no new translocations are involved.

All trisomic types occurring in the progenies of triploids should be obtainable directly from the diploid as a result of irregular chromosome disjunction. The only possible exception is the trisomic for chromosome 1·2, the pairing chromosome in *Oe. Lamarckiana*. This trisomic is expected in the progeny of triploids, but only following non-disjunction of the pairing chromosome in the progeny of the normal diploid. The complexes (either *velans* or *gaudens*) carrying 1·2 as a duplication together with the thirty-six complexes listed in table 1 are the only 8-chromosome complexes that can be produced by triploids. It might be supposed that 8-chromosome complexes with net duplications other than those already listed might occur, but it is impossible to construct such complexes from the chromosomes present in the triploid form, in which no chromosomes not represented in the diploid form are to be found. For example, there is no 8-chromosome complex listed with the net duplication 3+5. To derive such a complex from a triploid form it would be necessary to recover two chromosomes containing end 3 and two with end 5. These must be 3·4 and 5·8 of *velans* and *3·9* and *5·6* of *gaudens,* since there is no chromosome 3·5 present. Each of the remaining chromosome arms must be represented once and only once to give a viable 8-chromosome complex. Ends 1 and 2 will be represented by chromosome 1·2. End 7 must be represented by chromosome *7·11* of *gaudens*, since 6·7 of *velans* would make the complex a net duplication for end 6 in addition to ends 3 and 5. End 10 must be represented by *10·13* of *gaudens* since 9·10 of *velans* would again produce a further duplication (for end 9). This leaves ends 12 and 14 to be represented and there is no single chromosome in either *velans* or *gaudens* carrying these two arms. Hence it is not possible to have an 8-chromosome complex with a net duplication for arms 3 and 5, unless a new translocation occurs. Similar arguments show that no net duplications other than those listed in table 1, except 1·2, can arise directly from triploid *Lamarckiana.*

TRISOMIC TYPES FROM THE 8-CHROMOSOME COMPLEXES

Were it not for the zygotic lethals carried by certain chromosomes of *velans* and *gaudens*, each of the thirty-six 8-chromosome complexes listed in table 1 should function in two distinct trisomic types, one in conjunction with the normal *velans* complex and the other with *gaudens*. As it is, these characteristic zygotic lethals must greatly reduce the number of trisomic forms obtainable.

The particular trisomic types to be expected cannot be definitely determined until the loci of the zygotic lethals are established. RENNER (1933) has shown that the *velans* zygotic lethal is carried either in chromosome 5 8 or in 6 7, but until the chromosome arm in which it is carried is established we shall not know which chromosome of *gaudens* carries the normal allelomorph of this lethal. The situation is further complicated by our present inability to determine definitely all the chromosomes making up the *gaudens* complex. If *gaudens* actually carries the chromosomes tentatively ascribed to it above, the normal allelomorph of the *velans* lethal may be in any one of chromosomes *5 6*, *7·11*, *8 14*. The *gaudens* zygotic lethal could then be shown to be carried in one of chromosomes *3 9*, *4 12*, *10 13*, or the 11-arm of *7 11*, since none of the *gaudens* chromosomes present in ^{h}*deserens* carries the *gaudens* lethal; but here again the chromosome identifications in ^{h}*deserens* depend in part upon those tentatively ascribed to *gaudens*. The normal allelomorph of the *gaudens* lethal would then be in one of the *velans* chromosomes 3 4, 9·10, 11·12, 13 14.

Without knowing the exact positions of the lethals in the chromosomes, however, it is still possible to determine the number of different types of trisomics that can be obtained. In table 2 the possible lethal situations for each of the 8-chromosome complexes of table 1 are listed. From this table it can be seen that, regardless of the exact location of the lethals, some complexes will be effectively lethal-free and capable of functioning with both *velans* and *gaudens*. Others will carry one lethal, and not the normal allelomorph of that lethal, and consequently can function with only one *Lamarckiana* complex. Still others may carry both lethals and neither normal allelomorph and consequently will never occur in a viable trisomic type, since such complexes will be inviable with either normal *velans* or normal *gaudens*.

From table 2 it can be seen that complexes 1 to 6, in which there is a full complement of *velans* chromosomes with the extra chromosome from *gaudens*, will each form viable trisomic types with the normal *gaudens* complex regardless of the location of the *gaudens* lethal; and no matter which chromosome arm carries the *velans* lethal, one and only one of these complexes will also be capable of functioning with *velans*. The particular 8-chromosome complex that can function with both *velans* and *gaudens*,

TABLE 2

The distribution of lethals in the various 8-chromosome complexes for all possible positions of the lethals. Symbols: 1 = lethal present; c = lethal present but "covered" by presence of normal allemorph; o = lethal absent.

COMPLEX	*velans* LETHAL IN ARM 5	ARM 6	ARM 7	ARM 8	*gaudens* LETHAL IN ARM 3	ARM 4	ARM 9	ARM 10	ARM 11	ARM 12	ARM 13
1	1	1	1	1	c	o	c	o	o	o	o
2	1	1	1	1	o	c	o	o	o	c	o
3	c	c	1	1	o	o	o	o	o	o	o
4	1	1	c	1	o	o	o	o	c	o	o
5	1	1	1	c	o	o	o	o	o	o	o
6	1	1	1	1	o	o	o	c	o	o	c
7	o	o	o	o	c	c	1	1	1	1	1
8	c	o	o	c	1	1	1	1	1	1	1
9	o	c	c	o	1	1	1	1	1	1	1
10	o	o	o	o	1	1	c	c	1	1	1
11	o	o	o	o	1	1	1	1	c	c	1
12	o	o	o	o	1	1	1	1	1	1	c
13	1	1	1	1	c	o	1	1	o	o	c
14	1	1	c	1	o	c	o	o	1	1	o
15	c	o	o	1	o	o	o	o	c	o	o
16	o	c	1	o	o	o	o	o	o	o	o
17	1	1	1	c	o	o	o	c	o	o	1
18	1	1	1	1	1	1	c	o	o	c	o
19	o	o	o	o	c	o	1	1	c	o	1
20	o	o	o	o	o	c	o	c	1	1	1
21	c	o	o	1	1	1	1	1	1	1	c
22	o	c	1	o	1	1	1	1	1	c	1
23	o	o	o	o	1	1	c	o	o	1	o
24	1	1	c	c	1	1	1	1	1	1	1
25	1	1	1	c	c	o	1	1	o	o	1
26	c	o	o	1	o	c	o	o	1	1	o
27	o	c	1	o	o	o	o	c	o	o	1
28	o	o	o	o	o	o	o	o	c	o	o
29	1	1	c	1	1	1	c	o	1	1	o
30	1	1	1	1	1	1	1	1	o	c	c
31	o	c	1	o	c	o	1	1	o	o	1
32	o	o	o	o	o	c	o	o	1	1	o
33	o	o	o	o	o	o	o	c	c	o	1
34	c	o	o	1	1	1	c	o	1	1	o
35	1	1	c	1	1	1	1	1	1	1	c
36	1	1	1	c	1	1	1	1	o	c	1

however, can be definitely determined only when the exact position of the lethal is known. Similarly, complexes 7 to 12 will each form viable zygotes with *velans* and one of them will also be capable of functioning with *gaudens*.

Among complexes 13 to 18, in which two of the ring-chromosomes have been derived from *gaudens* and the remainder from *velans*, there must be one complex which will be lethal with *gaudens*, but there will also be two complexes which will not be lethal with *velans*. Similarly, among complexes 19 to 24, five must be capable of functioning with *velans* and two with *gaudens*. Only four of complexes 25 to 30 can function with *gaudens*, but three must form non-lethal zygotes with *velans*, and similarly in complexes 31 to 36 there will be four which form viable products with *velans* and three with *gaudens*.

Hence, no matter where the characteristic zygotic lethals of *velans* and *gaudens* are located, there must be a total of forty-two different trisomic types resulting from the thirty-six complexes listed in table 1. These, together with the form trisomic for chromosome 1 2, represent all the trisomic types that can arise directly from either the diploid or triploid forms of *Oe. Lamarckiana*.

GENETIC AND CYTOLOGICAL CHARACTERISTICS OF THE DIFFERENT TRISOMICS

The forty-two different trisomics resulting from the thirty-six 8-chromosome complexes of tables 1 and 2 fall into six groups, or classes, each with its own peculiar cytological and genetic potentialities. All members of each group should have identical chromosome configurations, but they may be divided into sub-groups on the basis of the preponderance of *velans* or *gaudens* chromosomes.

Chromosome configurations

Group 1. To the first group of trisomics belong all those that have a complete set of the chromosomes normally present in *Oe. Lamarckiana* with one of the ring-chromosomes present as an extra chromosome. There are twelve possible trisomics of this sort. Each of complexes 1 to 6 (table 1) has a complete set of *velans* chromosomes plus one *gaudens* chromosome, and each will form a viable compound with the normal *gaudens* complex (table 2). Similarly, each of complexes 7 to 12 carries a complete set of *gaudens* chromosomes together with one from *velans*, and each forms a viable product with normal *velans*.

Since the chromosomal constitutions of these twelve trisomics are similar, any one may be used as an example to illustrate the meiotic configurations possible in this group. The trisomic "complex-3·*gaudens*" is chosen as such an example and the principal configurations are diagrammed in figure 7. The pair 1 · 2 must always be present, but the remaining chromosomes may be arranged in various ways. If all homologous

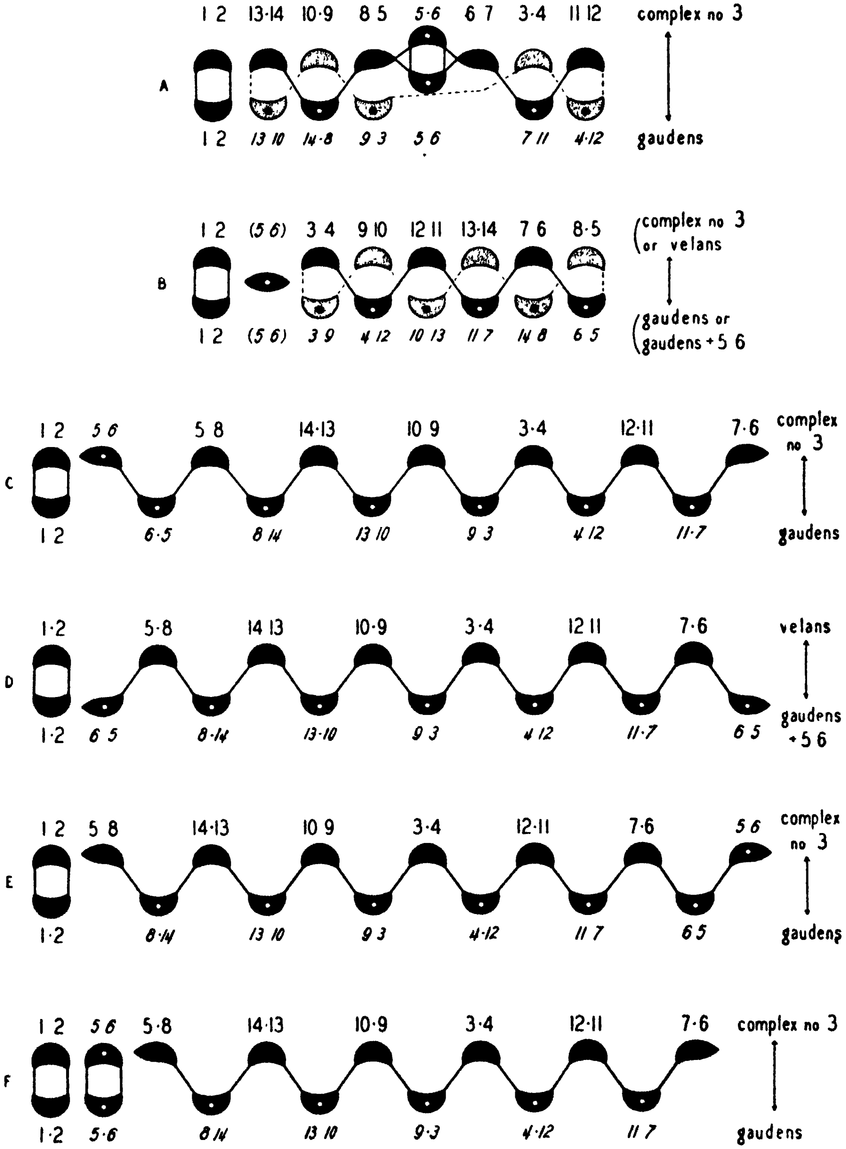

FIGURE 7.—Chromosome configurations in the trisomic "complex-3 · *gaudens*."

ends are associated, the two identical chromosomes of *gaudens* will constitute a pair intercalated in a closed ring by triple junctions (figure 7A). All other possible configurations have incomplete association of homologous ends. The extra chromosome may appear as a univalent with the remaining chromosomes forming a closed ring of twelve (figure 7B); or the extra chromosome may pair with its homolog leaving the remaining chromosomes to form an open chain of eleven (figure 7F); or all thirteen ring-chromosomes may be associated in three different ways to form an open chain of thirteen (figures 7C, D and E).

As an example of trisomics possibly belonging to this group, mut. *stricta* seems to be the best so far studied among the *Lamarckiana* derivatives. HÅKANSSON (1930) has observed a chain of thirteen and one pair in certain sporocytes and a chain of eleven and two pairs in others. *Oe. nutans* mut. *nanella* is a similar trisomic except that it has arisen from a diploid form with a ring of fourteen chromosomes. CATCHESIDE observed many different configurations: a chain of fifteen, a chain of thirteen and one pair free from the chain, a chain of thirteen with the pair attached at one end by a triple junction (CATCHESIDE, 1933 figs. 2b, 2e and 2f respectively).

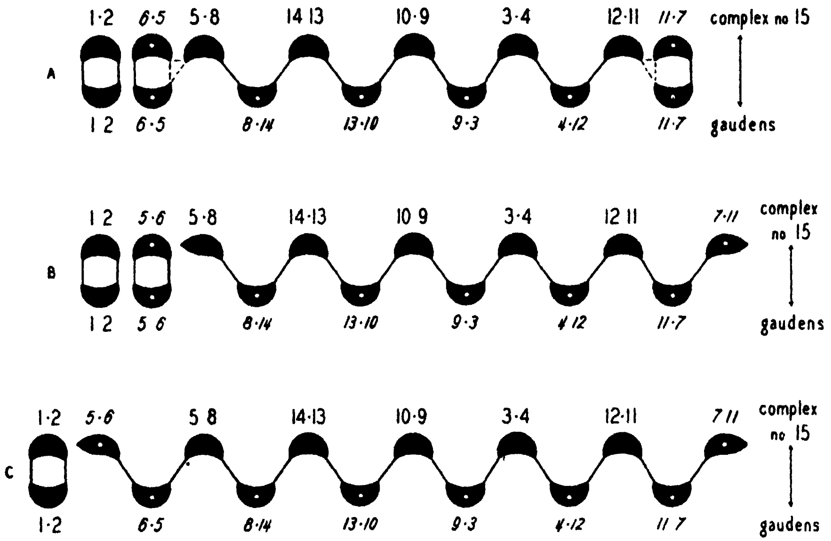

FIGURE 8.—Chromosome configurations in the trisomic "complex-15 *gaudens*."

Group 2. In the second group there are two chromosomes, in addition to 1 · 2, carried by both parental complexes, permitting a maximum of three pairs in meiotic prophase. In each of complexes 13 to 18 (table 1) there are two of the *gaudens* ring-chromosomes and five of these complexes (table 2) are viable with normal *gaudens*. Similarly, complexes 19 to 24 have each two of the *velans* ring-chromosomes and five of these complexes are viable with *velans*. The chromosome configurations in each of these ten trisomics will be sometimes a chain of thirteen and one pair (figure 8C), sometimes a chain of eleven and two pairs (8B), and other times a chain of nine and three pairs (8A). The extra chromosome pairs may be separate from the chain (8B) or either or both may be attached to the ends of the chain by triple junctions as illustrated in figure 8A.

Group 3. The trisomics belonging to the third group will always have two chromosome pairs but may have a maximum of four pairs, with the remaining chromosomes forming a chain in each instance. Figure 9 illustrates the configuration resulting from complete association of homologous ends, from which the other possible configurations may be inferred. The trisomics belonging to this group arise from unions of *gaudens* with the four complexes of 25 to 30 (table 1) which effectively lack the *gaudens* lethal (table 2) and from unions between *velans* and the four complexes of 31 to 36 which effectively lack the *velans* lethal.

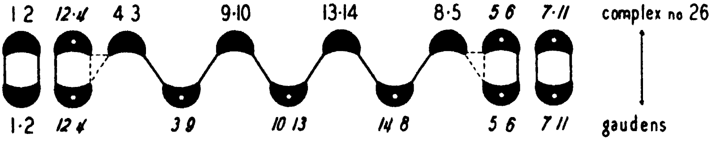

FIGURE 9.—Basic configuration in the trisomic "complex-26 *gaudens*"

Mutation *curta* may be an example of either group 2 or group 3. The configurations observed in this form were either a chain of nine and three pairs or a chain of eleven and two pairs (HÅKANSSON 1930).

Group 4. In addition to the four complexes of 25 to 30 which effectively lack the *gaudens* lethal, three of these complexes (including at least one which lacks the *gaudens* lethal) either have no *velans* lethal or have it covered by the normal allelomorph. These three complexes can thus produce trisomics from unions with normal *velans*. Similarly, three complexes of 31 to 36 can unite with *gaudens* to give viable products. These six trisomics belong to the fourth group in which the chromosome configuration varies between a chain of nine and three pairs, at one extreme, and a chain of five and five pairs at the other extreme (figure 10).

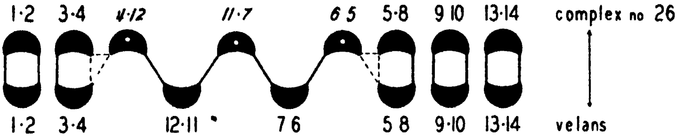

FIGURE 10 Basic configuration in the trisomic "complex-26 *velans*."

The trisomic form, mut. *oblonga*, studied by CLELAND (1923) may belong to this group. The observed configurations varied between a chain of nine and three pairs, and a chain of three and six pairs; a ring of five chromosomes was sometimes observed. This would seem to be too wide a range of variability for any one trisomic type. Unquestionably, several different trisomic types have been designated *oblonga;* DE VRIES (1929, p. 130) reports a trisomic, *persicaria*, which occurs in large numbers in the progeny of mut. *scintillans*, but which had previously been overlooked and may have been confused with *oblonga*. CLELAND does not state the origin of his material and it is not certain whether or not he was dealing with a single type.

Group 5. The trisomics belonging to the fifth group arise from the two complexes of 13 to 18 which effectively lack the *velans* lethal when these have united with *velans*, and from unions of *gaudens* with the two complexes of 19 to 24 which effectively lack that lethal. These four trisomics have configurations varying between a chain of seven and four pairs and a chain of three and six pairs as extremes (figure 11).

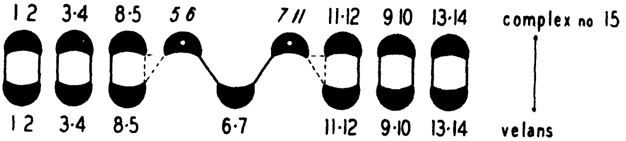

FIGURE 11.—Basic configuration in the trisomic "complex-15 · *velans*."

Group 6. The remaining two trisomics should have chromosome configurations varying between a chain of five and five pairs, at one extreme, and seven pairs and a univalent at the other (figure 12). One of these arises from a union of *velans* and that complex of 1 to 6 which carries the normal allelomorph of the *velans* lethal, and the other from a union of *gaudens* with the complex among 7 to 12 which has the *gaudens* lethal effectively suppressed.

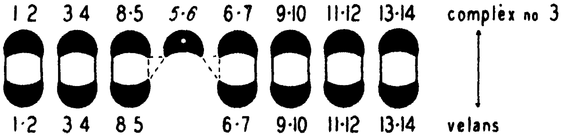

FIGURE 12.—Basic configuration in the trisomic "complex-3 *velans*."

Breeding behavior

Group 1. The type of segregation occurring in the progenies of the various trisomics can be inferred from the chromosome configurations characteristic of each. In the trisomics belonging to the first group, as outlined above, the chromosomes may be oriented in several different ways on the meiotic spindle. Following the diakinesis configuration of a ring of twelve chromosomes, a pair and a univalent (figure 7B), the six *velans* chromosomes of the ring together with one member of the pair, 1·2, will pass to one pole, and the six *gaudens* chromosomes of the ring together with the other member of the pair will pass to the opposite pole, whenever the chromosomes separate in the normal, zigzag manner. The extra, univalent chromosome (*5 6* of *gaudens* in the example illustrated) should pass to either pole at random. Hence, following this configuration, four products should be recovered in approximately equal frequencies: *velans; velans* plus the extra chromosome; *gaudens* plus the extra chromosome; and *gaudens*.

There are three different arrangements of chromosomes which result in the diakinesis configuration having a chain of thirteen and one pair. Regular chromosome disjunction following two of these arrangements (figures 7C and 7E) yields two products only: *velans* plus the extra chromosome; and *gaudens*. From the other arrangement (figure 7D) the products are: *velans;* and *gaudens* plus the extra chromosome. This configuration can thus produce the same four products as the preceding, but they are not expected to occur in equal frequencies. The exact frequencies cannot be predicted, but the extra chromosome would be expected to accompany *velans* more often than not in the example illustrated.

In all instances in which the extra chromosome has paired with its homolog (as in figures 7A and 7F), the extra chromosome must invariably accompany one particular complex, and this must be the *velans* complex in all trisomics of this group in which the extra chromosome came originally from *gaudens* (as in example, figure 7); whereas in those trisomics in which the extra chromosome originaly came from *velans* it must regularly accompany the *gaudens* complex. Hence, in any one particular trisomic, the four types of gametes produced should not occur in equal frequencies. In trisomics in which a *gaudens* chromosome is present as the extra chromosome, the products occurring in the higher frequency will be: *velans* plus the extra chromosome; and *gaudens*. In trisomics in which a *velans* chromosome is the extra chromosome, the more frequent products will be: *velans;* and *gaudens* plus the extra chromosome.

The inbred progenies of all trisomics of this group should contain typical diploid *Lamarckiana*, from a union of *velans* with *gaudens*, as well as the parental trisomic type, which can arise either from *velans* plus the extra chromosome when fertilized by *gaudens*, or from *gaudens* plus the extra chromosome fertilized by *velans*. These trisomics which regularly throw the diploid form have been called "dimorphic" mutations by DE VRIES. The following examples will serve to illustrate this type of segregation:

cana selfed: 49 per cent *Lamarckiana*, 35 percent *cana*, 16 percent mutations; n = 180.
pulla selfed: 80 percent *Lamarckiana*, 15 percent *pulla*, 5 percent mutations; n = 661.
liquida selfed: 76 percent *Lamarckiana*, 16 percent *liquida*, 8 percent mutations.; n = 507
cucumbis selfed: 89 percent *Lamarckiana*, 11 percent *cucumbis;* n = 420

The data quoted are all from DE VRIES, the first three from the 1925 paper, the last from the 1929 paper.

The product formed when a *velans* gamete fertilizes a gamete carrying *velans* plus the extra chromosome will be lethal except in the one trisomic in which the extra chromosome is that one from *gaudens* which carries the normal allelomorph of the *velans* lethal. In that particular trisomic, one of the two trisomics belonging to group six should appear in the inbred progeny in a relatively high frequency. If the trisomic in figure 7 is one of this sort, the trisomic in figure 12 should appear in its progeny. It is possible that the so-called accessory form, *oblonga*, appears in the inbred progeny of *scintillans* in this manner. DE VRIES (1913) reported the following segregation in inbred *scintillans:* 66 percent *Lamarckiana*, 21 percent *scintillans*, 12 percent *oblonga*, 1 percent mutations; n 1259. Similarly, the product formed when a *gaudens* gamete meets *gaudens* plus the extra chromosome will be inviable except in the one trisomic in which the extra chromosome is that one from *velans* which carries the normal allelomorph of the *gaudens* lethal.

The fact that the extra chromosome accompanies one complex more often than the other can be determined only in outcrosses. When the trisomic has been used as the female parent, the frequencies in which the extra chromosome appears in the *velans* and *gaudens* twins gives a direct measure of this preference. The following data from DE VRIES (1913) may be taken as an illustration:

OUTCROSS	DIPLOIDS WITH		TRISOMICS WITH		TOTAL
	VELANS PERCENT	GAUDENS PERCENT	VELANS PERCENT	GAUDENS PERCENT	
lata×*Hookeri*	43	39	17	1	1004
lata×*Cockerelli*	48	21	31	0	1389
lata×*chicaginensis*	55	21	23	1	1093

From these data it can be seen that the extra chromosome of *lata* goes most often with *velans*. In the haploid eggs of a trisomic of this sort, *gaudens* should be more frequent than *velans*, were it not that in the eggs of *Lamarckiana* itself, *velans* is much more frequent than *gaudens*. In outcrosses of *Lamarckiana* to the same pollen parents, DE VRIES (1916) obtained 82 percent *velans* twins and 18 percent *gaudens* twins. In *Oe. Lamarckiana* the frequencies of *velans* and *gaudens* megaspores must be equal, yet most of the functioning eggs in these hybrids of DE VRIES are *velans*, indicating that the *velans* members of the megaspore tetrads develop into embryo sacs more often than the *gaudens* members (RENNER 1921). In the trisomic *lata*, there should be an excess of *gaudens* megaspores produced, but if the *velans* megaspores are more likely to develop into embryo sacs, the functioning eggs carrying *velans* might still be in excess. The ratio of functioning eggs in *Lamarckiana* was 4.6 *velans* to 1 *gaudens*, and in *lata* 1.9 to 1. The relatively higher proportion of *gaudens* eggs in *lata* must indicate that *gaudens* megaspores are produced in excess of *velans*.

There should be the same difference in the frequencies of *velans* and *gaudens* in the microspores, and, since the 8-chromosome complexes are inviable in the pollen, the frequencies in which the two normal complexes are produced should be directly determinable from outcrosses in which the trisomics are used as pollen parents. Unfortunately, different outcrosses of *Lamarckiana* (DE VRIES 1913) show as great divergences in the *velans-gaudens* ratio as do outcrosses of the different trisomics to the same female parents. Hence it is unwise to attempt to distinguish between the sub-groups from such data alone.

Trisomic for chromosome 1 2. The trisomic carrying a member of the paired chromosome, 1·2, of *Oe. Lamarckiana* as the extra chromosome should resemble the members of group 1 in its breeding behavior. Since the extra chromosome may accompany either *velans* or *gaudens*, this trisomic should also throw diploid *Lamarckiana* in its inbred progeny. However, there should be no preference in the distribution of the extra chromosome, and *velans* and *gaudens* twins should appear in the same frequencies as in diploid *Lamarckiana*. This form can be easily recognized by its chromosome configuration which should be a ring of twelve and a trivalent group.

Groups 2 to 6. The trisomics belonging to the remaining five groups can be distinguished from those just described in that following self pollination they should breed true for the trisomic condition and never segregate diploid *Lamarckiana*. These trisomics are classed as "sesquiplex" mutations by DE VRIES.

Each trisomic belonging to groups 2 to 6 is made up of one normal *Lamarckiana* complex, either *velans* or *gaudens*, and an 8-chromosome complex in which there is a mixture of *velans* and *gaudens* chromosomes. As can be seen from figures 8 to 12 only the two parental complexes can result from regular chromosome disjunction following any configuration that the diakinesis chromosomes may assume. In the examples in figures 8 and 9, a complete set of *gaudens* chromosomes is always recovered at one pole and the eight chromosomes recovered at the other pole are in part *velans* and in part *gaudens;* in the examples in figures 10 to 12, the recovered products are *velans* and a mixed, 8-chromosome complex. Since the two parental complexes alone are recovered, these forms must breed true (homozygous *velans* and homozygous *gaudens* are lethal as in the diploid). *Oblonga, albida, candicans,* etc., are examples of true-breeding trisomics.

Outcrosses in which these trisomics are used as the female parents segregate into two types: a trisomic arising from the 8-chromosome complex, and either the *velans* or the *gaudens* "twin" characteristic of similar *Lamarckiana* outcrosses. The following examples from DE VRIES (1913 and 1923, respectively) illustrate this type of segregation:

	trisomics	*velans*-twin	*gaudens*-twin
oblonga×*chicaginensis*	19%	81%	0
delata×*chicaginensis*	46%	0	54%

Since the extra-chromosome complexes are not transmitted through the pollen, the functioning pollen of these trisomics is either all *velans* or all *gaudens*, depending upon which normal complex is present (DE VRIES 1913 and 1923).

Irregular disjunction in trisomics

The nature of the progenies of inbred trisomics as discussed above deals only with the products of regular disjunction. Irregular distributions of chromosomes in the first meiotic anaphase should occur in trisomic forms as well as in the normal diploid. In the trisomics, however, there is not ordinarily a closed ring made up of an even number of chromosomes, but an open chain in which there is an odd number of chromosomes. Hence it is possible for two adjacent chromosomes to pass to one pole with all remaining chromosomes separating in the regular, zigzag manner, as illustrated in figure 13B, with no necessity for a second, compensating irregularity such as is required in a closed ring of chromosomes.

Whenever there is one set of irregularly disjoining chromosomes in the chain of a trisomic, the products must be one 8-chromosome complex and one 7-chromosome complex (figure 13B). The complex receiving eight chromosomes will have a complete set of chromosome ends and in addition will have two ends in duplicate. The net duplication in this 8-chromosome product, however, will not ordinarily be the same as that in the parental 8-chromosome complex, but will be identical with some other 8-chromosome complex produced by *Lamarckiana* (table 1). There will be a different mixture of *velans* and *gaudens* chromosomes in the 8-chromosome products following irregular disjunction (compare figures 13A and 13B). On the other hand, the product receiving seven chromosomes, following irregular disjunction, will have a mixture of *velans* and *gaudens* chromosomes in which there will be a net deficiency for one chromosome arm (13 in the diagram) and duplication for another (arm 6 in the example illustrated). The seven chromosome products of irregular disjunctions of this sort must consequently be inviable.

Since irregular chromosome disjunction in trisomics results in 8-chromosome complexes different from the parental 8-chromosome complexes, new trisomic forms should be expected in the inbred progenies of practically all trisomics. These "new" trisomics, however, will be identical with certain of those produced directly from *Oe. Lamarckiana*, and if the chromosome constitutions of the trisomics were definitely established, it would be possible to predict just which trisomics should appear in the progeny of any particular one.

The number of trisomic types appearing in the progeny of any particular trisomic depends upon the number of different positions in the open chain at which irregularities in disjunction may occur. Consequently trisomics belonging to the different groups (pp. 210 214) should differ in the number of trisomic types produced.

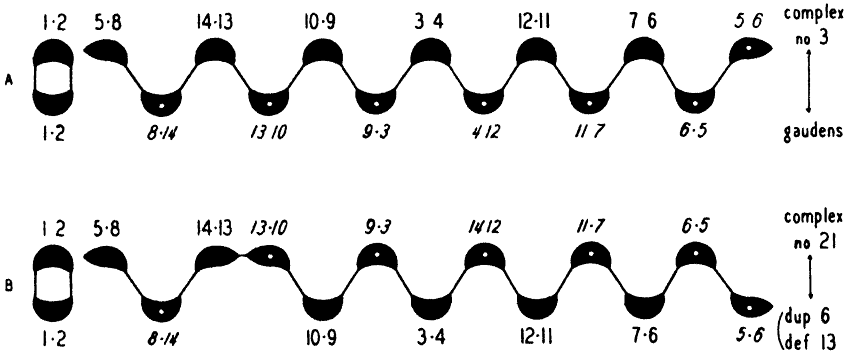

FIGURE 13.—Regular and irregular chromosome disjunction in the trisomic "complex-3 *gaudens*." Regular disjunction (A) yields the two parental complexes, irregular disjunction (B) results in a different 8-chromosome complex and an inviable 7-chromosome complex.

Each trisomic belonging to the first group should have eleven different trisomic types appearing in its progeny as a result of irregular distributions in the open chain of chromosomes. The two members of the group which have the normal allelomorphs of both zygotic lethals in the 8-chromosome complexes, however, should each produce seventeen different trisomic types. These are the numbers of trisomic types that should be produced in fairly high frequencies, but each of the trisomics of this group may occasionally have a ring of twelve chromosomes identical to the ring in *Lamarckiana* (see figure 7B), hence any of the trisomics produced directly from the diploid form may also occur in the progeny of each of the trisomics belonging to group 1, but many of these would be expected in very low frequencies.

The trisomics belonging to the remaining five groups always have open chains and never closed rings. The number of different trisomic types expected in the progenies of these groups are four for each member of group 2, three for group 3, two for group 4, and one for group 5. The two members belonging to group six should breed true and never throw other trisomic types. Trisomics belonging to the first group should give rise to "secondary" trisomics belonging to all six groups, those belonging to the second group can given rise to "secondary" types belonging to groups 3, 4, 5 and 6, trisomics of the third group to 4, 5 and 6, and so on.

The frequencies in which the different trisomics appear in the progeny of any particular trisomic should be higher than in the progeny of diploid *Lamarckiana*. In the diploid form, about half of the irregular distributions should result in products having each seven chromosomes (see p. 203), whereas any irregularity in the open chain of the trisomic must result in one 8-chromosome product. The published data seem to support this expectation.

DISCUSSION

In the foregoing account an attempt has been made to show how an application of the translocation interpretation to observed cytological behavior can account for the genetic and cytological characteristics of the trisomic derivatives of *Oe. Lamarckiana.* Wherever possible, specific examples from the literature have been quoted to illustrate the different points. There remain certain general and specific attributes of the trisomics which need further consideration.

Frequency of appearance of trisomics. Forms which have since been shown to be trisomics made up 1.2 percent of the progeny (which totaled 53,500) in DE VRIES' original inbred line of *Oe. Lamarckiana* (DE VRIES 1906). It has been shown above that trisomics are expected in the progeny of this species following certain types of irregularities in the zigzag arrangement of chromosomes in first meiotic anaphase. CLELAND (1929a) and CLELAND and OEHLKERS (1930) have shown that in 10 to 20 percent of the pollen mother-cells the chromosomes are distributed eight to one pole and six to the other. If such irregularities are correspondingly frequent in the megaspore mother-cells, 8-chromosome complexes should function as eggs sufficiently often to account for the frequency of trisomics observed by DE VRIES.

Number of different trisomics produced. A rather casual review of the literature shows that probably more than thirty distinct trisomics have been obtained from *Oe. Lamarckiana.* In the preceding analysis it was shown that forty-two or forty-three distinct types were expected. The remarkable thing is that so many have been recorded since many of them are nearly identical in external appearance (see DE VRIES and GATES 1928, DE VRIES 1929). A detailed cytological and genetic study would probably show that more than one type has been included under a particular name (see discussion of *oblonga* and *pulla* below).

Classification of trisomics. DE VRIES and his students have proposed two classifications for the trisomic derivatives of *Oe. Lamarckiana*. One of these classifications (DE VRIES and BOEDIJN 1923, DULFER 1926) is based on the assumption that, since the haploid number of chromosomes is seven, there should be seven "primary" trisomics in this species. The other trisomics are called "secondaries" because they have occurred principally in the progeny of the primaries, from which they are considered to differ by relatively few genes, as a possible result of crossing over (DE VRIES 1929). The seven groups are:

1. *lata* group: *semi-lata*, *albida*, *flava*, *delata*, *subovata*, *sublinearis*, *latifolia*, *synedra*, *planaria;*
2. *scintillans* group: *oblonga*, *aurita*, *auricula*, *nitens*, *distans*, *linearis*, *persicaria*, and some others,
3. *cana* group: *opaca*, *candicans*, *tardescens;*
4. *liquida* group: *cucumbis*, *lingua*, *plana;*
5. *spathulata* group: *hamata*, and perhaps others;
6. *pallescens* group: *lactuca;*
7. *pulla* group.

Though this classification is based upon a false interpretation, there must be other reasons why certain of the trisomics are sufficiently similar in phenotypic appearance to warrant grouping them together. Different trisomics will be *homozygous* for the same chromosome of *velans* or of *gaudens*, or for more than one chromosome, and each would be expected to have the characteristics determined by those particular chromosomes. Hence it is not surprising that many different trisomics have many phenotypic characteristics in common.

The other classification advanced by DE VRIES is based entirely on the breeding behavior. Two main groups are recognized, the dimorphic mutations which continually throw diploid *Lamarckiana* in their progenies, and the sesquiplex mutations which breed true. The latter are subdivided into a group having *velans* pollen and one having *gaudens* pollen. The dimorphic trisomics could be subdivided on the basis of the origin of the extra chromosome: if it comes from *velans*, most of the pollen and haploid eggs will be *gaudens*, and if the extra chromosome is from *gaudens*, the majority of the haploid gametes will be *velans*. As shown above (p. 214) there should be twelve different "dimorphic" trisomics, or thirteen if the trisomic for chromosome 1·2 is included. The trisomics ascribed to this group are: ***pulla***, ***cana***, ***ablata*** and ***cucumbis***, each with the extra chromosome from ***velans;*** ***lata***, ***scintillans*** and ***lingua***, each with the extra chromosome from ***gaudens;*** and ***liquida***, ***pallescens***, ***spathulata***, ***lactuca***, ***hamata*** and ***superflua***, for which there are not enough data to indicate the origin of the extra chromosome. The data on which this grouping is made are from DE VRIES (1913, 1916, 1925, 1929) and BOEDIJN (1925).

The true breeding "sesquiplex" trisomics which carry a normal *velans* complex are: *albida*, *oblonga*, *candicans*, *auricula*, *aurita*, *persicaria*, *opaca*, *cinerea*, *tardescens* and *planifolia* (DE VRIES 1913, 1923, 1929). Those having a normal *gaudens* complex are: *nitens*, *distans*, *flava*, *delata* and *diluta* (DE VRIES 1923, 1924). There are many other sesquiplex trisomics for which there are insufficient data to indicate their constitutions.

Trisomics involving new translocations. The discussion in the earlier parts of this paper has been confined to trisomics made up entirely of chromosomes normally present in diploid *Oe. Lamarckiana*. There are certain trisomic derivatives of *Lamarckiana*, however, which correspond to the tertiary trisomics of Datura (BELLING 1927) in that certain of the *Lamarckiana* chromosomes have been altered by translocation. The *pulla* studied cytologically by HÅKANSSON (1928) was found to have a ring of six chromosomes, three pairs and a trivalent group. The small ring of chromosomes must have arisen by a new translocation such as is responsible for the appearance of the so-called half-mutants (DARLINGTON, 1931). HÅKANSSON's *pulla*, however, must be different from the form studied genetically by BOËDIJN (1925) since the former could not segregate typical *Lamarckiana*. The *oblonga* in which CLELAND (1923) observe a closed ring of five chromosomes must again represent a new translocation, in this instance involving the "extra" chromosome, as in the tertiaries of BELLING. Such trisomics as *nitens* and its derivatives, *diluta* and *distans*, which originated from the cross *Lamarckiana*×*blandina*, must have chromosomes with different arrangements of homologous ends from that occurring in *Lamarckiana*, but the translocations in these instances took place in the formation of the *blandina* complex.

Data inconsistent with the interpretation. It was suggested earlier that the "accessory" trisomic *oblonga* which is produced in large numbers by the "dimorphic" trisomic *scintillans*, has two complete sets of *velans* chromosomes and only the extra chromosome from *gaudens*. Designating the extra chromosome as "g," the four types of egg cells possible in *scintillans* are *velans*, *gaudens*, *velans*+g and *gaudens*+g; and the functioning pollen is *velans* and *gaudens*. Then, following self pollination, *velans*×*gaudens* and *gaudens*×*velans* produce typical *Lamarckiana; velans*+g×*gaudens* and *gaudens*+g×*velans* reproduce *scintillans; velans*+g×*velans* results in *oblonga;* and the other possible combinations are lethal.

Data summarised from DE VRIES (1913, 1925, 1929) show about 19 percent *scintillans* and 11 percent *oblonga* in inbred cultures of *scintillans* (n = 2466, which includes data from the cross *scintillans*×*nanella*). In outcrosses to forms with *velans* pollen only there should be fewer *scintillans* since this type will result from only one type of egg, *gaudens*+g, which is relatively infrequent (see p. 215) and there should be more *oblonga*, since all eggs of the constitution *velans*+g should produce this form. In the cross *scintillans*×*oblonga* (DE VRIES 1913) there were 1 percent *scintillans* and 18 percent *oblonga* (n = 63); and in the cross *scintillans*×^{b}*albicans*·*velans* (DE VRIES 1929) there were 8 percent *scintillans*, 10 percent *oblonga* and 9 percent *persicaria* (n = 650). In both crosses the pollen was entirely *velans* and the data support the interpretation. In crosses to *gaudens* (or *rubens*) pollen, on the other hand, no *oblonga* should be produced, since that form arises only when a *velans*+g egg is fertilized by *velans*. The only eggs functioning in this cross are *velans* and *velans*+g, of which the latter should be more frequent (see p. 215). In the cross *scintillans*×*biennis* (all pollen *rubens*), there were 59 percent *scintillans* and 2 percent *oblonga* in a total of 300 plants (DE VRIES 1913). The high frequency of *scintillans* in this cross agrees with the interpretation, but the appearance of a few *oblonga* individuals cannot be accounted for.

Mutation *oblonga* should produce two types of eggs, *velans* and *velans*+g. In outcrosses to *Oe. Lamarckiana* as the pollen parent, *Lamarckiana*, *oblonga* and *scintillans* should be produced, the last named from *velans*+g ×*gaudens*, but apparently *scintillans* does not occur in such crosses. In a total of 244 individuals, DE VRIES (1913) reported 91 percent *Lamarckiana*, 7 percent *oblonga* and only 2 percent of "mutations" which were not specifically mentioned. In outcrosses to *Oe. biennis* in which all the pollen is *rubens* (= *gaudens*), all trisomics produced should be *scintillans* and never *oblonga*. In one such cross (n = 63) there were no trisomics, and in another (n = 87) 40 percent of the plants were recorded as *oblonga* and another 25 percent as *oblonga*-dwarfs. These results cannot be accounted for by the interpretation outlined above.

For most of the other trisomics, outcross data are too meagre to be used in testing the interpretations placed upon them.

CONCLUSIONS

All the more general genetic and cytological features of the trisomic derivatives of *Oe. Lamarckiana* are made more understandable when considered in relation to the translocation hypothesis. At the present time, however, it is impossible to account definitely for certain specific features of individual trisomics. Detailed cytological studies of particular trisomics and their hybrid-trisomic progenies could doubtless resolve the remaining problems. It should also be possible to determine the homologies of all *gaudens* chromosomes from cytological studies of outcrosses of the various trisomics to the standard "tester" races.

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THE DIFFERENTIATION OF EYE PIGMENTS IN DROSOPHILA AS STUDIED BY TRANSPLANTATION

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INTRODUCTION

PROMINENT among the problems confronting present day geneticists are those concerning the nature of the action of specific genes—when, where and by what mechanisms are they active in developmental processes? Despite the recognized importance of such questions as these, relatively little has been done toward answering them, a situation not at all surprising considering the difficulty of getting at these problems experimentally. Even so, promising beginnings are being made; from the gene end by the methods of genetics, and from the character end by biochemical methods. Probably the one factor which has played the most significant role in retarding progress in this field is the fact that relatively little is known from a developmental point of view about those organisms that have been studied most thoroughly from the genetic point of view, and, on the other hand, little is known genetically in those organisms that have been most studied from the developmental point of view. One of the two obvious (and alternative) ways of overcoming this difficulty would be to study development in a genetically well known organism. Drosophila, with its numerous mutant types, offers a favorable opportunity for a study of this kind. Several facts have led us to begin such a study on the differentiation of eye color pigments. Many eye color mutants are known, pigments have many advantages for chemical studies, and interactions between tissues of different genetic constitutions with respect to eye pigmentation are already known from studies of mosaics.

In this paper we shall present the detailed results of preliminary investigations (EPHRUSSI and BEADLE 1935a, 1935b, 1935c; BEADLE and EPHRUSSI 1935a, 1935b) which we hope will serve to point out the lines along which further studies will be profitable.

MATERIAL AND METHODS

The technique used in making transplantations in Drosophila has been described elsewhere (EPHRUSSI and BEADLE 1936). In brief, the desired organ or imaginal disc, removed from one larva, the donor, is drawn into

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[1] Work done at the Institut de Biologie physico-chimique, Paris and at the Station Biologique de Roscoff.

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a micro-pipette and injected into the body cavity of the host. As a rule, operations were made on larvae cultured at 25°C for three days after hatching from the eggs. At this time they are ordinarily about ready to pupate. Some of the stocks developed at slower rates than others, and larvae from these were sometimes used on the fourth day after hatching. In most cases the host larvae pupated within 24 hours after the operation. It is clear, from the above, that the stage of development at the time of operations was not controlled in a very precise way. However, since repetition of experiments at different times and, in some cases, with quite different stocks have given consistent results, we can be reasonably sure that the small differences in stage of development which may have existed between host and implant have not played any significant part.

The reasons for the choice of that stage of development reached shortly before puparium formation as "standard" for the studies reported here are largely those of convenience. At this time the optic discs are of a convenient size for transplantation, injections are readily made, and the host larvae require no more food.

As will be discussed below, implanted optic discs develop in a manner somewhat different from that characteristic of the same disc in its normal position. Because of this, it is not always desirable to compare the pigmentation of an implanted eye with that of a normal one. By dissecting the two eyes, normal and implanted, and observing fragments of the pigmented tissue, one can usually make a good comparison. However, to avoid all difficulty, which becomes important where slight differences are involved, we have practically always made comparisons only between implanted eyes. Thus, a vermilion eye disc implanted in a claret host gives rise to an eye with vermilion pigmentation. This conclusion is reached by comparing the implanted eye with an implanted eye known to be vermilion, obtained by implanting vermilion discs in vermilion larvae. Further comparisons with wild type and with claret control implants enable one to say definitely that the eye in question is vermilion, not wild type and not claret.

List of mutants

A list of the eye color mutants used in the studies reported in this paper is given together with their standard symbols. These mutant types and the genes which differentiate them from wild type will be referred to by symbol only. Other mutant genes were also carried by certain of the stocks used. These are indicated in the tables by symbol only since they presumably have no bearing on the results. These symbols are used generally in Drosophila work; their significance can be found in MORGAN, BRIDGES and STURTEVANT (1925).

bo — bordeaux
bw — brown
ca — claret
car — carnation
cd — cardinal
cl — clot
cm — carmine
cn — cinnabar
g^2 — garnet-2
Hn^r — Henna-recessive
lt — light
ma — maroon
p^p — peach
pd — purpleoid
pn — pruné
pr — purple
ras — raspberry
rb — ruby
se — sepia
sed — sepiaoid
sf^2 — safranin-2
st — scarlet
v — vermilion
w — white
w^a — apricot
w^e — eosin

DEVELOPMENT OF IMPLANTED EYES

When an eye transplant is made, the eye disc is injected into the body cavity of the host larva. The implanted disc continues development in the body cavity, and at maturity of the host usually comes to lie in the abdominal cavity. Occasionally, it may lie in the thorax but such cases are exceptional. The location of the implanted eye in the adult fly seems to be determined by purely mechanical factors; it is pushed into that part of the body cavity of the developing individual where the normal organs are least crowded. Usually injections are made toward the posterior end of the larva, but they have also been made near the anterior end, and this seems to have no effect on the final position of the eye. The implanted eye may lie just under the body wall of the adult fly where it is readily visible in the living fly, or it may lie deeply imbedded, in which case it may not be visible without dissection or clearing.

Very often the implanted eye becomes attached to other organs during its development. In females, it is often attached to one of the ovaries. This appears to be brought about mainly by the growth of tracheal tubes. In males the implanted eye may be attached to a testis. Males with an implanted eye sometimes have one testis which retains the ellipsoid shape which is characteristic of a testis at a much earlier stage of development. Such "inhibited" testes may have their sheaths normally pigmented but whether they contain viable spermatozoa is not known.

An implanted eye, which has developed within the body cavity of the host, is inverted as compared with an eye in its normal position. The normal eye has the shape of the head of a mushroom, the outer surface of the eye being represented by the top or convex surface of the mushroom head. An implanted eye disc is detached from its optic ganglion and, after development, its curvature is reversed in such a way that the facets are on the inside and the basement membrane on the outer convex surface. In other respects implanted eyes appear to be perfectly developed and differentiated; particularly, there seems to be no difference in the pigmentation of an implanted and a normal eye.

The optic and antennal imaginal discs in the larval stage are attached to each other. In removing an optic disc for transplantation, the antennal disc is usually left attached and implanted with the optic disc. This is not necessary but is done in routine procedure because it facilitates handling the discs and in most experiments does no harm. In special experiments where it may be desirable to do so, it is easy to remove the antennal disc and implant the optic disc alone. If the antennal disc is not removed and is not injured during dissection, it develops with the implanted eye and gives rise to an antenna, complete with an arista, attached to the eye by the chitinous head parts mentioned below. In most instances antennae developing with implanted eyes are normally everted.

The optic disc gives rise also to certain head parts when it is implanted, and presumably also in its development in the normal position. The exact extent of these head parts which arise from the optic disc has not been determined but they completely surround what would normally be the periphery of the eye and have normally developed bristles. As the developed implanted eye is inverted, these chitinous head parts form a kind of rim around the concave facet-side of the eye with the bristles on the inside.

In very exceptional cases an implanted eye disc may give rise to an external eye. This has happened only four times in about 1200 cases. In one of these, the eye was nearly normal, the facets were on the exterior convex surface, and there was a normally developed antenna attached to the eye by chitinous head parts. In all four cases the supplementary eye was attached to the abdominal wall of the adult fly, presumably at the point of injection. These cases are unusual and probably arise when the optic and antennal discs "plug," in a special way, the hole through which the pipette was inserted.

EXPERIMENTAL RESULTS

Because of the rather complex interrelations of the different types of data to be presented in this paper, they cannot be discussed efficiently until all the data have been presented.

In the following tables the various sex combinations of implant and host are given. In only one case, which will be specifically mentioned, does the sex of either the donor or the host appear to influence the result.

Mutant eye discs in wild type hosts

As a beginning in the study of the differentiation of eye pigment of implanted eyes, it is desirable to know how many eye color mutants are autonomous in their pigment development when implanted in wild type hosts. For the late larval stage, with which we are chiefly concerned in

TABLE 1

Data on the differentiation of mutant eye implants in wild type hosts Eye color mutant symbols are distinguished from symbols of incidental mutants present in the stocks by being printed in italics. In this and following tables, under the heading "number of individuals," are given the four sex combinations and the total in the following order: female in female, male in female, female in male, male in male, and total.

IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT	IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT
bo	+	3, 1, 1, 1, 6	?	*pd*	+	2, 0, 2,* 2; 6	*pd*
bw	+	4, 2, 2, 0; 8	*bw*	y *pn*	+	1, 1, 4, 0; 6	*pn*
ca	+	7, 0, 5, 2; 14	*ca*	b *pr*	+	4, 0, 1, 0, 5	*pr*
car	+	5, 0, 6, 0; 11	*car*	sc *ras*	+	2,‡ 0, 0, 0, 2	*ras*
cd	+	4, 0, 0, 0; 4	*cd*	*rb* cv	+	2, 0, 3, 0; 5	*rb*
cl	+	2, 2, 0, 1; 5	*cl*	*se* wo	+	5, 6, 5, 4, 20	*se*
cm	+	1, 0, 2, 1; 4	*cm*	sr *sed*	+	2, 2, 0, 6; 10	*sed*
cn	+	5, 3, 2, 0, 10	+	tk sf^2 abb	+	0, 2, 1, 1; 4	sf^2
cn	+/*v*	2, 0, 2, 0; 4	+	*st*	+	3, 0, 0, 2; 5	*st*
g^2	+	1, 1, 0, 0, 2	g^2	*v*	+	11, 6, 8, 5; 30	+
jv Hn^r h	+	0, 0, 1, 0, 1	Hn^r	*v*	+/*v*	1, 0, 0, 0; 1	+
lt c	+	0, 1, 2, 1; 4	*lt*	*w*	+	1, 1, 1, 0; 3	*w*
ma	+	2, 2, 2, 2; 8	*ma*	w^a	+	0, 0, 3, 0; 3	w^a
p^p	+	2, 0, 1, 1, 4	p^p				

* One fly in this class had an implanted eye with wild type pigmentation—presumably because of a mistake in the selection of the donor

‡ One host in this class dissected as mature pupa

TABLE 2

Data on the differentiation of wild type eye implants in eye color mutant hosts. Arrangement as in table 1.

IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT	IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT
+	*bo*	0, 0, 0, 1; 1	?	+	p^p	3,* 2,* 0, 0; 5	+
+	*bw*	2, 1, 1, 0; 4	+	+	*pd*	2, 0, 6, 0; 8	+
+	*ca*	2, 2, 2, 0; 6,* 1*; 13	*ca*	+	y *pn*	2, 4, 0, 1; 7	+
+	*car*	1, 0, 1,‡ 0; 2	+	+	b *pr*	3, 1, 4, 1; 9	+
+	*cd*	4, 1, 0, 0; 5	+	+	sc *ras*	1, 1, 1, 1; 4	+
+	*cl*	2, 2, 5, 1; 10	+	+	*rb* cv	1,‡ 0, 2, 1; 4	+
+	*cm*	2, 1, 0, 0; 3	+	+	*se* wo	5, 1, 8, 1; 15	+
+	*cn*	3,* 1,* 0, 0; 4	+	+	sr *sed*	2, 3, 0, 0; 5	+
+	g^2	1,* 0, 0, 0, 1	+	+	tk sf^2 abb	1, 0, 1, 2; 4	+
+	jv Hn^r h	4, 1, 1, 6; 12	+	+	*st*	1, 1, 1, 0; 3	+
+	*lt* c	2, 1, 0, 0; 3	+	+	*v*	3, 2, 5, 2; 12	+
+	*ma*	2, 2, 5, 7; 16	+	+	*w*	6, 0, 0, 0; 6	+

* Sex of donor not determined.

‡ Host dissected as mature pupa.

this paper, the data on this point are presented in table 1. These data show that most of the eye color mutants are autonomous in their pigmentation. The only clearly exceptional cases are those of *v* and *cn*. When implanted in wild type or in heterozygous *v*, the pigmentation of both of these is that characteristic of a wild type eye. In the case of *bo*, the result is not clear because the visible difference between an implant with *bo* pigmentation and one with wild type pigmentation is very slight. This is also true of the two eye color types as seen in normal eyes. Special experiments using other mutants as "intensifiers" of the difference between *bo* and wild type will probably be necessary to determine the behavior of *bo*.

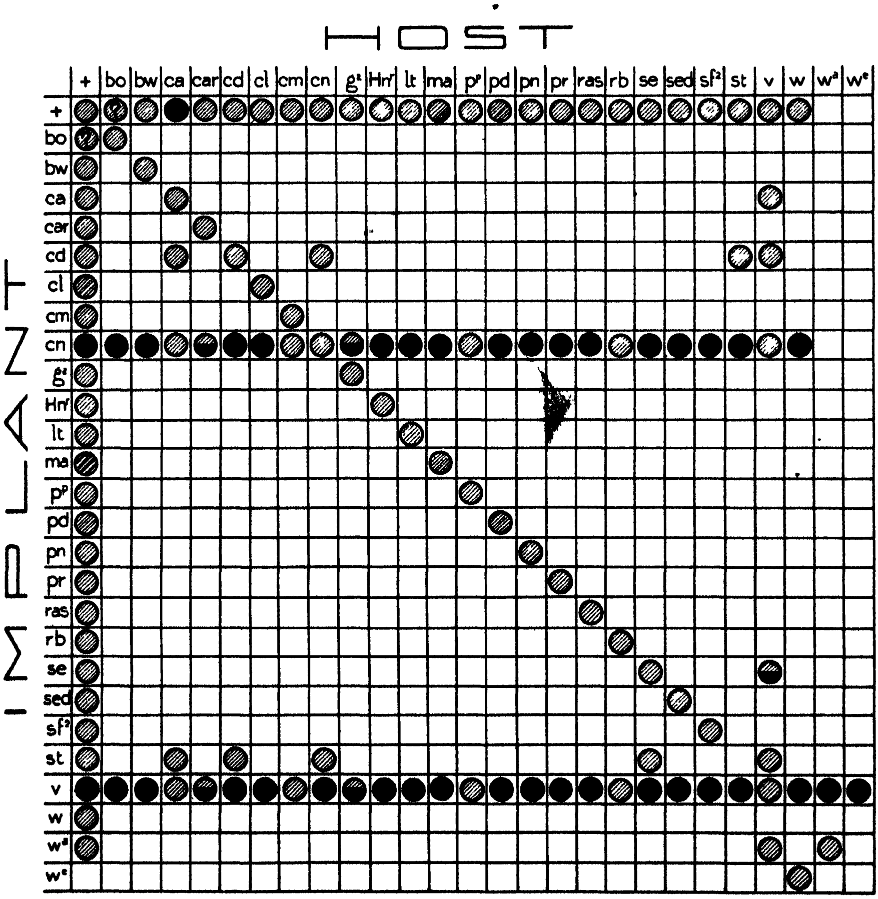

FIGURE 1.—Diagrammatic representation of the results of eye transplants. Shaded circles indicate autonomous development of the pigmentation of the implant. Black circles indicate non-autonomous development of pigmentation. Circles half black and half shaded indicate non-autonomous development of such a nature that the resulting implant is intermediate in color between two controls.

Wild type discs in mutant hosts

Knowing the behavior of the various mutant eye color discs implanted in wild type hosts, the reciprocals of these offer points of interest. The data are summarized in table 2.

It is evident that a wild type disc gives rise to an eye with wild type pigmentation when implanted in any of the mutants except *ca* and possibly *bo*. As in the reciprocal transplant, the result with *bo* is not clear. The significance of this exceptional behavior of + in *ca* transplants will be discussed later.

Vermilion discs in mutant hosts

In the case of a *v* disc implanted into a wild type host, the developing eye is affected by the host in such a way that the final pigmentation is like that of a wild type eye. Before discussing the factor responsible for this change in more detail and its relation to the factor responsible for the fact that a *cn* eye disc implanted into a wild type host develops wild type pigmentation, data should be considered which bear on the question of whether other eye color mutants have anything to do with this "body-to-eye" phase of the *v* reaction. This question can be answered by implanting *v* eye discs into hosts which differ from wild type by various eye color mutants. Such data are given in table 3.

TABLE 3

Data on the differentiation of v eye implants in eye color mutant hosts. Arrangement as in table 1.

IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT	IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT
v	*bo*	0, 0, 0, 1; 1	+	*v*	*pd*	2, 1, 1, 0; 4	+
v	*bw*	7, 0, 2, 1; 10	+	*v*	y *pn*	2, 0, 1, 1; 4	+
v	*ca*	4, 4, 3, 2, 3,* 2*; 18	*v*	*v*	b *pr*	2, 0, 1, 1; 4	+
v	*car*	5, 0, 2, 1; 8	Interm	*v*	sc *ras*	0, 1, 0, 1; 2	+
v	*cd*	2, 1, 4, 1; 8	+	*v*	*rb* cv	2,‡ 1, 1, 0; 4	*v*
v	*cl*	2, 3, 1, 0; 6	+	*v*	*se* wo	4, 4, 6, 1; 15	+
v	*cm*	7, 0, 1, 2; 10	*v*	*v*	sr *sed*	0, 1,‡ 0, 0; 1	+
v	*cn*	6, 1, 3, 3; 13	+	*v*	tk sf^2 abb	3, 0, 0, 1; 4	+
v	g^2	2, 0, 3, 2; 7	Interm.	*v*	*st*	3, 0, 3, 2; 8	+
v	jv Hn^r h	1, 0, 2, 1; 4	+	*v*	*w*	8, 2, 2, 1; 13	+
v	*lt* c	2, 0, 0, 0; 2	+	*v*	w^a	0, 0, 0, 1; 1	+
v	*ma*	3, 0, 0, 3; 6	+	*v*	w^e	0, 1, 0, 0; 1	+
v	p^p	3,* 2,* 0, 0; 5	*v*				

* Sex of donor not determined.

‡ One host dissected as mature pupa.

These data show that, when implanted in certain mutant hosts (*bo*, *bw*, *cd*, *cl*, *cn*, Hn^r, *lt*, *ma*, *pd*, *pn*, *pr*, *ras*, *se*, *sed*, sf^2, *st*, and *w*), a *v* optic disc gives rise to a wild type eye; in others (*ca*, *cm*, p^p, and *rb*), it gives an eye with *v* pigmentation. In *car* and g^2 hosts, a *v* disc gives an eye with pigmentation intermediate between *v* and wild type. Discussion of these relations will be deferred until other evidence is considered.

Cinnabar discs in mutant hosts

Since a *cn* disc implanted in a wild type host gives a result of the same type as the comparable implant of a *v* disc, namely, a wild type eye, the same question arises concerning *cn* as the one stated above for *v*. Data showing the results obtained by implanting *cn* eye discs in eye color mutant hosts are given in table 4. The results, excluding *cn* and *v* hosts, are the same as those for *v*, that is, a *cn* disc gives a wild type eye in the same mutant hosts in which a *v* disc gave a wild type eye, and gives a *cn* eye in the same hosts in which a *v* disc gave a *v* eye. Table 3 shows that a *v* disc in a *cn* host gives a wild type eye. Table 4 shows that the reciprocal transplant does not give this result, that is, a *cn* disc in a *v* host gives a *cn* eye.

TABLE 4

Data on the differentiation of cn eye implants in eye color mutant hosts. Arrangement as in table 1.

IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT	IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT
cn	*bo*	1, 1, 2, 0; 4	+	*cn*	*pd*	1,‡ 0, 5, 1; 7	+
cn	*bw*	3, 0, 0, 3; 6	+	*cn*	y *pn*	3, 0, 0, 0; 3	+
cn	*ca*	2, 0, 1, 0, 3	*cn*	*cn*	b *pr*	0, 1, 2, 0; 3	+
cn	*car*	2, 0, 5, 3; 10	Interm.	*cn*	sc *ras*	0, 0, 0, 1; 1	+
cn	*cd*	3, 3, 2, 2; 10	+	*cn*	*rb* cv	1, 0, 2, 1; 4	*cn*
cn	*cl*	2, 0, 1, 2; 5	+	*cn*	*se* wo	3, 3, 2, 3; 11	+
cn	*cm*	1, 2, 3, 1; 7	*cn*	*cn*	sr *sed*	0, 3, 2, 0; 5	+
cn	g^2	5, 1, 1, 2; 9	Interm.	*cn*	tk sf^2 abb	3, 0, 3, 0; 6	+
cn	jv Hn^r h	1, 0, 3, 0; 4	+	*cn*	*st*	3, 2, 2, 2; 9	+
cn	*lt* c	0, 0, 2, 1; 3	+	*cn*	*v*	2, 4, 5, 1, 12	*cn*
cn	*ma*	1, 2, 1, 1; 5	+	*cn*	*w*	0, 2, 1, 0, 3	+
cn	p^p	5,* 3,* 0, 0; 8	*cn*				

* Sex of donor not determined.

‡ Host dissected as mature pupa.

Experiments concerning v, cn, and ca

From the data present above, it is seen that, in the cases of *cn* in wild type, *v* in wild type, and wild type in *ca*, the developing eye implant is influenced in its pigmentation by something that either comes or fails to come from some part or parts of the host. Just what this is, whether or not, for example, it is of the nature of a hormone, we cannot yet say. We shall therefore refer to it by the noncommittal term "substance."

Certain obvious questions at once arise concerning the substances concerned in these three cases. For example, is there only one substance? If not, are the different substances related and in what way? What is their relation to the genes concerned in their production? Before attempting to discuss these and related questions, we shall consider additional data which bear on the problem.

Behavior of v in combination with other eye color mutants

By studying the differentiation of pigment in implants which differ from the host tissues by two eye color characters, one autonomous, the other non-autonomous in development, it might be possible to learn something about the interaction of the genes concerned. Data of this nature are summarized in table 5A for the combinations of *v*, $w^a v$ and *v car*. It is seen that the behavior of *v* is here the same as that observed in transplants in which *v* is the only mutant gene concerned. Likewise, *car* and w^a behave in the same way as in simple transplants involving only these mutant genes. This result tells us only that, so far as its behavior in transplants goes, the interaction of the *v* allelomorph with *car* or w^a plus the normal allelomorphs of all the other genes concerned with eye pigmentation is not different from its interaction with car^+ or w^+ under the same conditions. The same kind of result was observed by STURTEVANT (1932) in studies of early cleavage mosaics in *D. simulans* in which the individuals were made up of v^+g^+ and *v g* tissue; here the *v* character is, under certain conditions, not autonomous, but the *g* character is always autonomous.

The relation of Bar and vermilion

In studies of the differentiation of Bar (*B*) eye discs implanted in not-*B* hosts, it was observed that a v^+B disc implanted in a *v* host gives rise to a *B* eye[1] with *v* pigmentation.

This experiment was repeated several times varying both the *v* stocks used as hosts and the *B* stocks which furnished the implants. The result was in all cases the same, indicating that the *B* gene, in addition to influencing the size of the eye in a characteristic way, has an effect closely related to the *v* reaction. The data from the various experiments involving the *v* and *B* mutants, as well as appropriate controls are given in table 5B. It is seen that only in case the host is *v*, does the *B* implant develop *v* pigmentation. An eye disc heterozygous for the *B* gene implanted in a *v* host gives an eye with wild type pigmentation. It follows that, whatever its action may be, the *B* gene effect is recessive in this interaction with *v*. These results suggested that the condition of some process in the *B* eye

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[1] It is clear that a *B* disc implanted in a not-*B* host is *B* but whether or not there is any modification of the *B* character such as is observed in mosaics (STURTEVANT, 1932), we have not yet determined.

TABLE 5.

Data on various eye implants. Explanations in text.

IMPLANT	HOST	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT
		PART A	
sc *v* f *car*	+	14, 7, 4, 2; 27	*car*
+	sc *v* f *car*	1, 0, 2, 0; 3	+
sc *v* f *car*	*v*	2, 2, 1, 0; 5	*v car*
v	sc *v* f *car*	2, 0, 4, 1; 7	*v*
sc *v* f *car*	*cn*	1, 3, 3, 2; 9	*car*
$w^a v$	+	0, 0, 1, 1; 2	w^a
+	$w^a v$	6, 4, 2, 0; 12	+
$w^a v$	*v*	0, 0, 1, 1; 2	$w^a v$
v	$w^a v$	0, 2, 0, 4, 6,* 3*; 15	*v*
$w^a v$	*cn*	0, 0, 2, 0, 2	w^a
cn	$w^a v$	4, 5, 1, 2, 12	*cn* (host eyes w^a)
$w^a v$	wa^3	0, 0, 7, 0; 7	w^a
		PART B	
B	y *v* f	0, 0, 3, 0; 3	*v B*
B	*v*	2, 0, 1, 1, 4	*v B*
g^2 f *B*	*v*	0, 0, 3, 2, 5	*v* g^2B
B/+	*v*	3, 2, 0, 0, 5	*B*/+
B	+	0, 0, 3, 0, 3	*B*
B	*B*	0, 0, 0, 1; 1	*B*
se wo	*v*	6, 1, 5, 5; 17	*se* Interm *v* (sex diff.-text)
		PART C	
cd	*cn*	2, 0, 0, 0; 2	*cd*
cd	*st*	3, 2, 6, 2; 13	*cd*
cd	*v*	2, 0, 3, 0; 5	*cd*
st	*cd*	0, 4, 1, 3; 8	*st*
st	*cn*	4, 3, 2, 3; 12	*st*
st	*v*	3, 0, 2, 0; 5	*st*
st	*se* wo	3, 0, 1, 0, 4	*st*
B	*st*	0, 0, 1, 2; 3	*B*
g^2 f *B*	*st*	0, 0, 1, 0; 1	g^2B
se wo	*cn*	4, 1, 1, 1, 7	*se*
cd	*ca*	3, 2, 5, 1; 11	*cd*
st	*ca*	1, 1, 0, 0; 2	*st*

* Sex of donor not determined.

disc at or after the time of transplantation might be retarded relative to the state of other developmental reactions, and led to experiments in which eye discs from young wild type larvae were implanted in older *v* larvae.

In table 6 data are given from transplants of this kind. In the first experiment, only two transplants were successful in the sense that the implanted discs gave rise to differentiated eyes. Here the age difference between implant and host, at the time of transplantation, was about 28 hours. One of the two implanted eyes showed *v*-like pigmentation, the other more nearly wild type pigmentation. Unfortunately, in this experiment, there were no satisfactory controls. Later, an experiment was made in which young wild type discs were implanted in older *v* larvae, and at the same time, for a control, wild type discs of the same age and from the same culture dish of larvae were implanted in older wild type larvae. The data (table 6) show that, with an age difference of about 28 hours, the wild type discs implanted in *v* hosts did indeed give eyes with pigmentation approaching in color that of control *v* in *v* implants. The wild type in wild type controls with a similar age difference gave eyes with pigment of the same type as did known wild type control implants. In all cases the young discs implanted in older hosts gave rise to eyes markedly smaller than implanted eyes from transplants where little or no age difference exists between implant and host.

TABLE 6

Data on the differentiation of wild type eye discs from young larvae implanted in older v larvae. Arrangement under heading "Number of individuals" same as in previous tables.

IMPLANT		HOST			
CONSTITUTION	AGE AFTER HATCHING (HRS.)	CONSTITUTION	AGE AFTER HATCHING (HRS.)	NUMBER OF INDIVIDUALS	PHENOTYPE OF IMPLANT
+	44 to 48	*v*	80±	1, 1, 0, 0; 2	♀ *v* (?) ♂ + (?)
+	43 to 46	*v*	80±	3, 1, 3, 1, 8	Interm between + and *v*
+	44 to 47	+	80±	4, 0, 1, 0; 5	+

From the data so far discussed, it might be assumed that the difference between *B* in *v* and wild type in *v* transplants is determined merely by the smaller size of the *B* implants. The behavior of young wild type implants in older *v* hosts could then be interpreted in the same way. But there are two arguments against this interpretation. In the first place, we have often obtained, from wild type in *v* transplants where there was no age difference, small fragments of eyes resulting from breakage of the disc during the operation of transplantation. In all cases these "small eyes" had wild type pigmentation. Many of these fragments were smaller than the eyes obtained in the "young in old" transplants. Furthermore, it is known from mosaics that small patches of v^+ tissue in an otherwise *v* eye have wild type pigmentation (STURTEVANT, unpublished). The second argument is one from analogy with the behavior of *se* in *v* transplants discussed below, in which there was little or no age difference between implant and host, but in which the implanted eyes were intermediate between *v* and v^+ (actually intermediate between *se* and *v se*, since *se* is autonomous in its development). Here the implanted eyes were "normal" in size since the *se* gene does not affect eye size.

Actually, then, it appears probable that the behavior of *B* in *v* implants will find its explanation in terms of the states of certain eye reactions, influenced by the *B* gene, relative to the states of certain developmental reactions in other parts of the organism. Such a situation can, of course, following GOLDSCHMIDT, be expressed in terms of rates of certain eye reactions relative to the rates of other developmental reactions. What the nature of this eye reaction (or reactions) might be, we have, at present, no way of knowing. We shall return later to a consideration of its possible relation to the action of the *v* gene.

The experiments on *se* in *v* transplants mentioned above are summarized in table 5B. Actually these data are the result of three separate experiments, all of which gave the same result. Two *se* stocks were used, the second obtained by outcrossing the first to a *v* stock and recovering *se* flies in the backcross to the *se wo* stock. There was a definite difference between eyes developed from implants of discs from male and female donors; the male discs gave eyes with pigmentation more closely approaching *v se* control implants (*v se* in *v se*) than did female discs. Speculation concerning this effect of *se*, which may be of the same kind as the effect of *B*, will be more profitable when more data are at hand. The nature of the observed sex difference also needs further investigation.

Influence of eye implants on host eye pigmentation

In the above experiments in which w^av stocks were used, it was observed that w^av flies in which implanted *cn* eyes had developed, had normal eyes with w^a rather than w^av pigmentation. Since the w^av stock used had been recently made up, it was at first thought that this stock might not be pure. However, the same experiment was later repeated with adequate controls and the same result obtained. A *cn* eye implant, then, furnishes something to a w^av host fly which changes the course of eye pigment formation in such a way that the result is, in effect, v^+ and not *v* pigmentation. Since no such action of wild type or *cn* eye implants on the normal eyes of *v* hosts had been observed previously, a series of transplants was made to check this point carefully. The results were as follows:

Implanted eye disc	Host	Pigmentation of	
		Implant	Host
+	*v*	+	*v*
cn	*v*	*cn*	*v*
v	*v*	*v*	*v*
+	w^av	+	w^av
cn	w^av	*cn*	w^a
w^av	w^av	w^av	w^av

These results suggest two obvious questions. The first is, why are the eyes of a $w^a v$ host changed by *cn* eye implants to w^a (from v to v^+) while the eyes of a v host are unaffected by such an implant? This change in the $w^a v$ eyes seems to be complete in many cases, that is, the modified eyes show no difference from stock w^a flies. Hence it seems clear that the same proportionate change does not occur in the two cases, detectable in $w^a v$ and not in v hosts. A more probable interpretation assumes that a *cn* eye implant releases into the blood of the host a certain quantity of some substance, presumably the same as that which changes the pigmentation of a v implant in a wild type host, and that this substance is only sufficient in amount to result in the change of a limited amount of pigment from v to v^+. The $w^a v$ eyes have little pigment and this can all be changed, by the available substance, from v to v^+. The normal eyes of a v host, on the other hand, have such a large amount of pigment that the limited supply of substance does not produce a detectable change, even though it may result in a change of the same absolute amount of pigment as in the case of the eyes of a $w^a v$ host. This interpretation obviously can be tested by relatively simple experiments. In fact, we have already observed that, in case the *cn* eye implant is small, the change in $w^a v$ is not complete.

A second question that is apparent from these results is, why is a *cn* eye implant effective whereas a wild type implant has no effect? Both types of eye implants of course have the v^+ gene, and presumably the production of v^+ substance goes on in both. It seems from the data that the *cn* gene produces a change such that the substance in question is released from the implant.

In connection with the influence of an eye implant on the eye color of the host, it is known, from studies of w^+-w gynandromorphs in *D. simulans* (DOBZHANSKY 1931; STURTEVANT 1932), that rate of testis sheath pigmentation is correlated with the amount of w^+ eye tissue present. The substance responsible for the pigmentation of the testis sheath very probably is formed by w^+ eye tissue—if so, it must be able to diffuse from the eye.

Implantation of gonads

In his studies of v-v^+ early cleavage mosaics in *D. simulans*, STURTEVANT (1932) was able to demonstrate clearly a strong correlation between the autonomous or non-autonomous pigmentation of genotypically v eye tissue and the constitution of the gonads with respect to the v gene. Here, if both gonads are v^+ (and female), genetically v eye tissue show v^+ pigmentation in practically all instances. If, on the other hand, both gonads are v (and male), genetically v eye tissue shows v or intermediate pigmentation in all cases. We have pointed out in a preliminary paper (EPHRUSSI and BEADLE 1935a) that it is the constitution of the gonads with respect to the *v* gene and not with respect to sex that is important. In these experiments of STURTEVANT, there were some exceptions which led him to conclude that, in addition to the gonads, some other organ or part of the fly must be involved in the differentiation of *v* eye tissue in mosaics.

On the basis of STURTEVANT'S results we have made transplants of wild type ovaries in *v* hosts to see whether we could influence the pigmentation of the eyes of the host. Such ovary implants develop quite normally and are even capable of forming functional connections with the oviducts of the host (EPHRUSSI and BEADLE 1935b). The results of such experiments with ovary transplants, and which bear on the *v* case, are summarized in table 7.

TABLE 7

Data on transplants of non-v ovaries to v hosts.

CONSTITUTION		NUMBER OF DEVELOPED IMPLANT OVARIES	NUMBER OF INDIVIDUALS		PHENOTYPE OF HOST
IMPLANT	HOST		FEMALE	MALE	
+	*v*	1	17	7	*v*
ca	*v*	1	4		*v*
+	$w^a v$	1	29	5	$w^a v$
cn	$w^a v$	1	1		$w^a v$
+	*v*	2	5	2	*v*
+	*y v f*	3	2		*v*

It is seen that one or two wild type ovaries in a *v* male host or one, two, or even three such ovaries in a *v* female host, have no detectable effect on the *v* color of the eyes of the host. Likewise, neither an implanted wild type nor an implanted *cn* ovary has any influence on the normal eyes of a $w^a v$ host, male or female. These results, then, are entirely negative. Since in all these cases normal *v* ovaries or testes were present in the host, it could be argued that they account for the fact that implanted ovaries are without effect on the host eyes. However, this seems rather improbable as it would involve the assumption that the implanted ovaries produce the necessary substance but that something else produced by either *v* ovaries or *v* testes acts as an inactivating agent on the v^+ substance.

Taken in connection with the results of STURTEVANT which show quite definitely that wild type ovaries do have something to do with the production of the substance which changes the course of pigment formation in *v* eye tissue, our results only corroborate his conclusion that some other organ or part of the body plays an essential role in the production of this substance, i.e., gonads plus an unknown part of the body interact in its formation. Our studies give no clue as to what this unknown might be, but STURTEVANT has shown that it is not closely related in terms of cell lineage to any surface part of the body, and does not lie in the abdomen (1932).

These results of gonad transplantation in Drosophila show certain obvious differences from those obtained by CASPARI (1933) and KÜHN, CASPARI and PLAGGE (1935) in gonad transplants in *Ephestia kühniella*, likewise made in connection with studies on eye pigmentation. These workers have shown that wild type testes or ovaries implanted in larvae of the red-eyed mutant race *a*, modify the eye pigmentation toward wild type. Here, then, the substance concerned, which they refer to as a hormone, can evidently be formed by the gonads from a wild type race in the absence of other organs or tissues of a^+ constitution. In this case, the substance has an effect on pigmentation in several parts of the organism, in larval skin, larval eyes, eyes of the imago, and in the gonads themselves. The substance can evidently be produced in other parts of the body since a wild type brain implanted in an *a* host modifies, under certain conditions, the pigmentation of the host.

Special experiments with the v-like group of mutants

The four mutants, *v*, *cn*, *st*, and *cd*, are very much alike in their phenotypic appearance. Furthermore, SCHULTZ (1935) has shown that in the development of their pigmentation, they show rather marked similarities and, as a group, are distinct from other mutants. In fact, on the basis of these similarities, he was led to suggest that they might all be found to show the *v*-type of behavior in mosaics. It has already been shown that, although *v* and *cn* are not autonomous in their pigment development in certain kinds of transplants, *st* and *cd* do show autonomous development in eye transplants in wild type hosts. Because of the similarity of *st* and *cd* to each other and to *v* and *cn*, we have used them in certain transplants in which other mutants have not been used (table 5). These data need little discussion. It is evident that both *st* and *cd* show autonomous development of pigment in all the combinations in which they are involved.

It is clear that the *v*-like group of mutants is not homogeneous as regards developmental behavior. In this respect *v* and *cn* are obviously related but not the same, as will be pointed out in more detail below, and *st* and *cd* are different from either *v* or *cn*.

The ca case

As shown by the data already referred to, a wild type eye disc implanted in a *ca* host gives an eye with *ca*-like pigmentation. To account for this result, we must assume that in the development of wild type pigment something must come to the eye from another part or other parts of the body and that this substance is not formed in a fly homozygous for the *ca* gene. But the data given in tables 3 and 4 show that a *v* eye disc implanted in a *ca* host gives a *v* eye, i.e., not *v ca*, therefore ca^+, and that, similarly, a *cn* disc implanted in a *ca* host gives a *cn* ca^+ eye. Data given in table 5 show that a similar result is obtained if a *st* or a *cd* disc is implanted in a *ca* host, namely, a *st* ca^+ or a *cd* ca^+ eye results. Summary of these results:

+ disc implanted in a *ca* host gives a *ca* eye
v disc implanted in a *ca* host gives a *v* ca^+ eye
cn disc implanted in a *ca* host gives a *cn* ca^+ eye
st disc implanted in a *ca* host gives a *st* ca^+ eye
cd disc implanted in a *ca* host gives a *cd* ca^+ eye

In determining that the last four of these results were really *v*, *cn*, *st* and *cd* and not *v ca*, *cn ca*, *st ca*, and *cd ca* respectively, the appropriate double recessive controls were not available, but comparisons were made with *v*, *cn*, *st*, and *cd* control transplants and no differences could be detected. Since *v ca* and *st ca* are both known to be readily separable from *v* and *st* respectively, there is little chance of error in the determinations. The question, of course, is, why is the development of ca^+ pigmentation not autonomous in the first case listed and autonomous in the remaining cases studied? Possibly the four genes *v*, *cn*, *st* and *cd* act, in the implant, in such a way that no ca^+ substance is necessary to give ca^+ pigmentation; that is, a v^+ca^+ implant requires ca^+ substance from the host to develop ca^+ pigmentation, but a *v* ca^+ implant does not require this substance to develop *v* ca^+ pigmentation.

DISCUSSION

From the experimental results considered above, several hypotheses can be suggested concerning the nature of certain of the eye color mutants and the action of the genes which differentiate them from wild type. Alternative hypotheses are obviously possible, and it should be emphasized that those presented are tentative.

The vermilion character

Since the pigmentation of a genetically *v* eye can be modified to v^+ by transplanting it to a host which supplies it with what may be called the v^+ substance, it follows that *v* differs from wild type by the absence of this substance. Evidently there is no change in the *v* eye itself which prevents its pigmentation from assuming wild type characteristics. It follows that the mutation $v^+ \rightarrow v$ has resulted in a change such that v^+ substance is no longer formed. Since a *cn* eye disc implanted in a *v* host remains *cn*, the $v^+ \rightarrow v$ mutation has resulted also in preventing the formation of cn^+ substance. The v^+ gene plays an essential part in the formation of the v^+ and cn^+ substances, but it does not form them directly since any one of several other gene mutations (ca, cm, p^p, and rb) may result in the absence of them. According to this scheme, v^+ substance is necessary for wild type pigmentation. The question then arises, why does a wild type eye disc implanted in a v host, which can supply no v^+ substance, develop wild type pigmentation? Two answers are possible: either the v^+ substance has already acted at the time of transplantation or this substance is produced by the eye itself. The fact that, in mosaics, a small patch of v^+ eye tissue in an effectively v individual has wild type pigmentation (STURTEVANT, unpublished), shows that the first of these answers cannot be correct, for in this case, the v^+ tissue has been in a v tissue environment almost from the beginning of development. We must then conclude that the substance is produced in the eye itself. Actually we have been able to demonstrate that it is produced by a cn eye (modification of normal $w^a v$ eyes by an implanted cn eye). But, it may be asked, why was it not possible to demonstrate that it is produced by a wild type eye? The answer may be that the substance is produced but cannot get out of the eye, i.e., one of the effects of the cn gene is to make eye cells permeable to v^+ substance. The difference in behavior between a wild type and a B eye implanted in a v host may be accounted for by assuming that one of the effects of the B gene is to prevent the formation of v^+ substance in the eye, but not in other parts of the body. This assumption is not necessarily an alternative to the assumption previously suggested that the action of the B gene may be explained "in terms of the states of certain eye reactions, influenced by the B gene, relative to the states of certain developmental reactions in other parts of the organism." It may well be that it is the formation of v^+ substance that is retarded (in an extreme way) in the eye relative to its formation in other parts of the body. The "young in old" experiments can be formally explained in the same terms. In young wild type discs implanted in older v larvae, the time during which v^+ substance can be formed in the implanted eye is much reduced. In a similar way, in a sc eye, v^+ substance is formed in the eye at a rate so low that, when implanted in a host without v^+ substance, pigmentation intermediate between v^+ and v results.

The cinnabar character

The evidence for the existence of a cn^+ substance is the same in kind as that for v^+ substance. It is already evident and will be pointed out in more detail below that the cn^+ substance is different from the v^+ substance. By the same kind of arguments as were presented in the above discussion of the v character, it may be concluded that the mutation $cn^+ \rightarrow cn$ produces a change such that *cn*+ substance is no longer formed. According to this interpretation, as in the interpretation of *v*, it is assumed that a wild type eye produces *cn*+ substance in its own cells. This would account for the fact that a wild type eye implanted in a *cn* host gives wild type pigmentation.

The claret character

In contrast to the *v* and *cn* cases, two phases of the action of the *ca* gene can be distinguished. First, since a genetically wild type eye cannot develop wild type pigmentation unless some other part of the organism is *ca*+, it is concluded that a *ca*+ substance is necessary for the formation of wild type pigmentation. This is not formed in the eye itself but comes from some other part of the body. Secondly, since by supplying a *ca* eye with the necessary *ca*+ substance by implanting it in a wild type host, we do not produce a change to wild type pigmentation, it is postulated that there is a change in a *ca* eye of such a kind that the addition of *ca*+ substance is not sufficient to give wild type pigmentation.

Other eye color mutant characters

By implanting *v* and *cn* eye disc in other eye color mutant hosts, it has been demonstrated that the mutants *cm*, p^p, and *rb* are characterized by lack of both the *v*+ and *cn*+ substances. In these three mutant types, as in *ca*, there must be two phases of gene action, (1) the failure of the formation of the *v*+ and *cn*+ substances, and (2) an action in the eye itself, since supplying the two substances by transplantation does not produce a change. The genes *car* and g^2 must be placed in the same class, but in these two cases the formation of *v*+ and *cn*+ substances is not prevented but only limited.

The other mutants with which we have worked, *bo, bw, cd, cl,* Hn^r, *lt, ma, pd, pn, pr, ras, se, sed,* sf^2, *st,* and w^a are characterized by the presence of all the three substances postulated. It cannot be concluded that the normal allelomorphs of the genes differentiating these characters have nothing to do with the production of *v*+, *cn*+ and *ca*+ substances. There is no justification in assuming that, if a given gene concerned with the production of a substance such as we are considering, mutates, the particular mutant allelomorph resulting will be of such a nature as to result in the absence of the substance. KÜHN, CASPARI and PLAGGE (1935) come to such an unjustified conclusion with regard to the *t*+ gene in Ephestia.

Relation of the v+, cn+ and ca+ substances

It has been shown from the difference in reciprocal transplants between *v* and *cn* that the *v*+ and *cn*+ substances are different (BEADLE and EPHRUSSI, 1935a). At the same time, it was concluded from the fact that a *v* fly lacks both substances, that the two substances are related. This conclusion is corroborated by the more extensive data presented in this paper. The strongest indication that two substances are concerned is the fact that a *v* eye disc implanted in a *cn* host gives rise to an eye with wild type pigment. Two other facts strengthen the supposition of two substances: (1) A *B* eye disc implanted in a *v* host gives an eye with *v* pigmentation, but, implanted in a *cn* host, gives wild type pigmentation. (2) A *se* eye disc implanted in a *v* host gives a *se*, partially *v*, eye, but, implanted in a *cn* host, gives a straight *se* eye.

The fact that these substances, although not the same, are developmentally—and presumably chemically—related, is shown by the fact that, if a given mutant is characterized by the absence of one of these substances, it will probably be characterized by the absence of the other also.

Considering the relation of the ca^+ substance to the other two, it is clear that it is different from either for it may be present in the absence of both the others. The fact that the *ca* gene prevents the formation of all three substances (*v* or *cn* discs implanted in *ca* hosts are not modified in their pigmentation) indicates that ca^+ substance is related to the other two.

It may be asked whether, from the relations discussed above, anything can be inferred as to (1) how the v^+, cn^+, and ca^+ substances are related in terms of development, and (2) how the mutant forms of the genes known to be concerned with the production of the three substances produce their effects? A simple, and, it seems to us, plausible, hypothesis may be of help in answering these questions. Such an hypothesis assumes that the ca^+, v^+, and cn^+ substances are successive products in a chain reaction. The relations of these substances can be indicated in a simple diagrammatic way as follows:

$\rightarrow ca^+$ substance $\rightarrow v^+$ substance $\rightarrow cn^+$ substance

In such a scheme, we assume that:

1. The mutant gene *ca* in some way produces a change such that the chain of reactions is interrupted at some point prior to the formation of ca^+ substance; hence a *ca* fly lacks ca^+, v^+, and cn^+ substances.

2. Any one of the mutant genes *v*, *cm*, p^p, or *rb* results in a change such that the reaction or reactions leading from ca^+ substance to v^+ substance do not go on; hence the mutants *v*, *cm*, p^p and *rb* lack both v^+ and cn^+ substances but have ca^+ substance. The mutant genes *car* and g^2 slow down this step in the chain of reactions, hence *car* and g^2 flies are characterized by a reduced amount of v^+ and cn^+ substances. The mutant gene *B* interrupts this same step in the chain in the eye, but not in other parts of the body. The mutant gene *se* results in a change such that the ca^+ substance changes to v^+ substance at a reduced rate in the eye, but at a normal rate in other parts of the body.

3. The mutant gene *cn* stops a reaction essential for the change of v^+ substance to cn^+ substance; hence a *cn* fly lacks cn^+ substance but has the ca^+ and v^+ substances.

On the basis of the above scheme, the results of implanting *v* eye discs in *cn* hosts can be interpreted as follows: The implant produces no v^+ substance, and, because v^+ substance is an essential step in the formation of cn^+ substance, it likewise produces no cn^+ substance. The host can supply v^+ substance to the implant but cannot supply cn^+ substance. With v^+ substance supplied to the implant by the *cn* host, there is no block to the formation of cn^+ substance in the implant itself. The implant therefore develops wild type pigmentation in spite of the fact that normally neither the donor nor the host could have produced the cn^+ substance presumably necessary for the production of wild type pigment.

In a somewhat similar way, the results of transplanting *B* eye discs to *v* and to *cn* hosts can be interpreted. The *B* eye can form no v^+ substance. When transplanted to a *v* host v^+ substance cannot move to it from the host and the pigment developed is therefore *v*. Because of the absence of the prerequisite v^+ substance, the *B* eye normally does not itself produce cn^+ substance. But when a *B* eye disc is implanted in a *cn* host, the *B* implant is supplied with v^+ substance from the host and the reaction or reactions from v^+ to cn^+ substances can then go on in the implant itself and wild type pigment is produced.

The results of implanting *se* eye discs in *v* and *cn* hosts can be interpreted in an essentially similar way.

Eye color mutant groups

The eye color mutants in Drosophila can be grouped according to their phenotypic characteristics, since mutants differentiated by non-allelomorphic genes can look alike (MORGAN, BRIDGES, and STURTEVANT 1925). Recently SCHULTZ (1935) has extended this grouping by studying the time of appearance and the rate of formation of pigment, the distribution of pigment in the eye, and the interaction behavior of the different mutants. It is obvious that we can, on the basis of the results given above, classify the mutants with respect to the presence or absence of the three postulated substances. We may then ask if there is any relation between groups such as made by SCHULTZ and the classification according to these substances. If there is such a relation, it is not evident from the data at hand. As an example, the four mutants, *v*, *cn*, *st*, and *cd*, form one of SCHULTZ's groups but as we have seen, *v* lacks two substances, *cn* one, while *st* and *cd* have all three.

The above discussion, we hope, has served to indicate some of the possibilities in the application of the method of transplantation to the study of development in Drosophila. The extension of the studies of certain cases to other stages of development is indicated as a logical next step by which we can hope to get at such questions as concern the time of determination of characters and the time of action of genes associated with these characters.

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SUMMARY

Larval optic discs can be successfully transplanted from one larva to another. Such transplanted discs give rise to supplementary eyes, usually lying in the abdominal cavity of the adult fly, which differentiate normally except that they are inverted. The pigmentation of such eyes develops normally.

When optic discs of the mutants *cn* or *v* are implanted in wild type hosts, they give eyes with wild type pigmentation, i.e., under these conditions, the *cn* and *v* characters are not autonomous in their development. Under the same conditions, *bw*, *ca*, *car*, *cd*, *cl*, *cm*, g^2, Hn^r, *lt*, *ma*, p^p, *pd*, *pn*, *pr*, *ras*, *rb*, *se*, *sed*, sf^2, *st* and *w* eye discs implanted in wild type hosts show autonomous development of eye pigment.

In the reciprocals of the above transplants, wild type eye discs implanted in hosts of the mutants mentioned, wild type pigmentation of the implant results in all except one case, a wild type disc implanted in a *ca* host. In this one exception, a genetically wild type eye disc gives an eye with *ca* pigmentation, i.e., ca^+ does not show autonomous pigment development under these conditions.

If *v* eye discs are implanted in eye color mutant hosts, eyes with wild type pigmentation develop in *bo*, *bw*, *cd*, *cl*, *cn*, Hn^r, *lt*, *ma*, *pd*, *pn*, *pr*, *ras*, *se*, *sed*, sf^2, *st*, and *w* hosts, i.e., the *v* character is not autonomous in its development when a *v* eye is transplanted to any one of these hosts. But a *v* eye disc implanted in a *ca*, *cm*, p^p, or *rb* host gives an eye with *v* pigmentation, i.e., the *v* character is autonomous in these cases. It can be concluded that the autonomous or non-autonomous development of the *v* character is determined by the genetic constitution with regard to genes other than *v*, of the tissue environment in which the *v* eye develops.

Implanted in eye color mutant hosts other than *v* or *cn*, a *cn* eye disc behaves in the same way as does a *v* eye disc, showing autonomous pigment development in the same mutant hosts as does *v*, and non-autonomous pigment development in the same hosts as does *v*.

Reciprocal transplants involving *cn* and *v* do not give the same result; a *v* eye disc implanted in a *cn* host gives an eye with wild type pigmentation while a *cn* eye disc implanted in a *v* host gives an eye with *cn* pigmentation.

A *B* eye disc implanted to a *v* host gives a *B* eye with *v* pigmentation. This shows that the *B* gene has an effect on the eye somehow related to the effect of the *v* gene but not of such a nature as to modify the pigmentation of the eye in its normal position. This case shows that the autonomous or non-autonomous development of v^+ pigmentation in an implanted v^+ eye may be influenced by the genetic constitution, with respect to genes other than *v*, of the implant itself.

A genetically wild type eye disc from a young larva implanted in an older *v* host shows pigmentation intermediate between *v* and wild type. A *se* eye implanted in a *v* host likewise gives pigmentation of an intermediate nature with respect to the *v* character; here the eye is intermediate between *v se* and *se*. The possible relation of these cases to the *B* in *v* results is considered.

A *cn* eye implanted in a w^av host gives a *cn* eye, but the eyes of the host are modified from the w^av to a w^a phenotype.

Ovaries from wild type donors have been implanted in both male and female *v* hosts without any detectable change in the pigmentation of the host eyes.

From the cases of non-autonomous development of the pigmentation of implanted eyes considered in this paper, three substances are postulated, the v^+, cn^+, and ca^+ substances. Their interrelations and the conditions under which they are produced are discussed. A hypothetical scheme accounting for the production and relation of these three substances is suggested, and, in connection with this, questions concerning where and how certain genes might act are considered.

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THE DEVELOPMENT OF VESTIGIAL WINGS UNDER HIGH TEMPERATURE IN DROSOPHILA MELANOGASTER

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INTRODUCTION

THE PRESENT paper deals with three questions concerning the development of vestigial wings in Drosophila: First, where is the critical period during which the development of vestigial can be affected by high temperature (31°), the so-called temperature-effective period? Second, to what extent can the wing form be modified by temperature? And lastly, is the enlargement of the wing as a result of the temperature treatment due to the increase of size or number of cells?

The work of HARNLY (1930, 1932 and 1933), STANLEY (1928, 1931 and 1935), HERSH (1932) and others has already covered a considerable amount of ground in the study of the vestigial and temperature relationship in Drosophila. The present work, however, represents the beginning of a slightly different approach to the question and is concerned with a more direct embryological study than has hitherto been attempted.

THE TEMPERATURE-EFFECTIVE PERIOD

A pure vestigial stock of *Drosophila melanogaster* originally obtained from Columbia University, New York City, was used for the following experiments. The food was prepared from local material according to the formula adopted for this laboratory (LI 1930). Only two temperatures were employed to treat the vestigial flies: 25° and 31°C. For the lower temperature, we have used the cabinet gas-burning type of incubator designed by BRIDGES and LI (1932); for the high temperature, the only incubator available was of the water-bath type. During the years 1932 to 1934 the following experiments were performed.

High temperature treatment of pupae

In a number of cultures five pairs of strong and vigorous vestigial flies were allowed to breed. The larvae were raised in the 25° incubator and, as soon as puparia were formed, they were isolated within an hour. About 30 pupae thus isolated were put on a strip of moistened blotting paper and raised through the rest of their life cycle in a vial with a small amount of food to keep the paper continuously moist. These vials were then divided

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into two batches: "A" and "B." The one-hour old pupae in the vials of "A" series were allowed to start their development at 31°, but at each of the succeeding 6-hour intervals, were transferred to 25° for the rest of their life cycle. In the vials of the "B" series the pupae were allowed to begin their development at 25° and were subsequently transferred at the same time intervals to 31°. The purpose of the reciprocal transfers in the experiment was to narrow down from both ends of the pupal period the temperature-effective period for the high temperature. As controls, four cultures were tested simultaneously with the others. First, both larvae and pupae were raised at 25° (X1, table 3); second, the larvae were raised at 25°, but the pupae at 31° (X2); third, both larvae and pupae were raised at 31° (Y1); and lastly, the larvae were raised at 31°, but the pupae at 25° (Y2).

When the adults emerged from the pupal cases, they were etherized and their wings were measured under a binocular microscope with a standard micrometer. As a rule only the right wing of each fly was measured. However, when the latter was injured or folded, the left wing was then taken as a substitute, provided both wings were of approximately equal lengths. The results of the experiment are summarized in the following tables:

TABLE 1

The mean lengths in mm of vestigial wings of flies beginning their pupal period at 31° and transferred to 25° at the intervals indicated.

HOURS AT 31°	♀♀		♂♂	
	M	N	M	N
6	0.81	19	0.74	19
12	0.77	36	0.69	20
18	0.70	33	0.69	21
24	0.73	19	0.63	17
30	0.73	27	0.67	19
36	0.74	24	0.66	27
42	0.75	28	0.69	37
48	0.76	17	0.68	36
54	0.80	26	0.70	25
60	0.75	18	0.70	26
66	0.79	27	0.68	24
72	0.73	24	0.66	27
78	0.77	30	0.74	27

The results given in tables 1 and 2 show that the mean lengths of the vestigial wings of the treated flies are fairly uniform, which means that there is no particular period in the whole pupal stage that responds to high temperature treatment. Comparing with the 25° control (X1, table 3), it can be further shown that there is no significant increase in wing length as a result of high temperature. If there is any effect at all, high tempera-

TABLE 2

The mean lengths in mm of vestigial wings of flies beginning their pupal period in 25° and transferred to 31° at the intervals indicated.

HOURS AT 25°	♀♀		♂♂	
	M	N	M	N
6	0.68	15	0.64	11
12	0.71	15	0.61	22
18	0.79	19	0.68	17
24	0.76	17	0.70	21
30	0.73	20	0.72	30
36	0.78	19	0.72	42
42	0.78	36	0.71	25
48	0.79	20	0.74	25
54	0.78	28	0.73	32
60	0.82	17	0.72	25
66	0.79	30	0.69	24
72	0.86	28	0.71	21
78	0.83	27	0.75	30

TABLE 3

The mean lengths in mm of wings of all vestigial flies raised in both "A" and "B" series and in the controls

SERIES	♀♀		♂♂	
	M	N	M	N
"A"	0.75±0.01	328	0.69±0.01	325
"B"	0.78±0.01	291	0.71±0.01	325
X1	0.80±0.01	132	0.74±0.01	129
X2	0.78±0.01	12	0.71±0.01	15
Y1	1.06±0.04	32	1.63±0.05	25
Y2	1.05±0.01	124	1.45±0.02	122

ture tends to make the vestigial wings slightly shorter when the pupae are subjected to it, as indicated in table 3. When we compare the mean lengths of the vestigial wings of all the flies in both "A" and "*B*" series and those of the controls X1 and X2, it can be noted that when the pupae were raised at 31,° the wing length of the fly is consistently shorter than otherwise. The results in table 3 also show that when the larvae were raised at 31°, irrespective of treatment of the pupae, there is a great increase in wing length (Y1 and Y2, table 3). It follows therefore that a much more significant temperature-effective period is to be found in the larval period.

High temperature treatment of larvae

In order to locate this critical period in the larval stage by means of reciprocal transfers the larvae of various ages were exposed to 31°. This would naturally necessitate careful isolation of the newly hatched larvae, as it has been shown that there is a considerable amount of variation in the time of hatching of eggs isolated at the same time (LI 1927). For this purpose a large number of vials was prepared, in each of which a glass slide with a piece of blotting paper of corresponding size was placed. The blotting paper was first soaked in fermented banana juice and then a thin layer of banana agar food was carefully spread on it. Five pairs of strong parental vestigial flies were allowed to remain on the food while eggs were laid during a 6-hour interval in the 25° incubator. Slides with eggs on them were taken out of the vials and examined one by one under a binocula. The newly hatched larvae within the interval of one hour were isolated with a fine scalpel and transferred to a culture bottle, fifty larvae in each bottle. Thus they were all about one hour old and allowed to develop under almost identical conditions of food and amount of space. The culture bottles in duplicates were then treated as in the case of pupae in the earlier experiments, that is, reciprocal transfers between two temperatures 25° and 31°. As soon as the larvae pupated the bottles were all removed to 25° to let the flies finish the rest of the life cycle. Tables 4 and 5 give the results of the wing measurements of the treated flies.

In working through the data shown in the above two tables one may note that there is a great variability in the wing measurements of the individual flies as indicated by the high value of the coefficient of variabilities (C.V., tables 4 and 5). The percentages of mortality in most cases

TABLE 4

Mean lengths in mm of vestigial wings of flies beginning their larval period at 31° and transferred to 25° at the intervals indicated.

HOURS AT 31°	♀♀			♂♂			PERCENT MORTALITY
	M	C.V.	N	M	C.V.	N	
6	0.85±0.01	9.53	21	0.72±0.02	9.40	12	38.91
12	0.89±0.02	14.18	23	0.82±0.01	7.02	19	22.21
18	0.86±0.01	9.69	20	0.75±0.01	9.33	11	42.58
24	0.83±0.01	10.32	23	0.78±0.01	9.39	19	22.21
30	0.85±0.02	11.87	19	0.73±0.01	8.22	31	57.70
36	0.86±0.01	12.71	33	0.73±0.01	7.45	53	21.75
42	0.88±0.02	15.10	31	0.75±0.01	9.00	32	51.75
48	0.92±0.02	11.01	22	0.78±0.01	7.28	38	44.38
54	0.86±0.01	10.26	26	0.86±0.01	14.90	43	37.75
60	0.93±0.01	10.05	35	0.89±0.03	22.85	29	40.75
66	1.02±0.02	10.94	24	0.90±0.01	6.39	23	12.96
72	0.99±0.02	10.48	20	0.90±0.03	16.22	15	35.20
78	1.09±0.01	16.18	17	1.49±0.05	22.21	18	35.20
84	1.05±0.03	23.30	28	1.23±0.05	30.28	29	47.20
90	1.12±0.03	20.08	33	1.44±0.06	28.30	19	51.83
25° control	0.80±0.01	15.08	132	0.74±0.01	11.21	129	—
31° control	1.06±0.04	25.87	31	1.63±0.05	22.41	25	—

TABLE 5

Mean lengths in mm of vestigial wings of flies beginning their larval stage at 25° and transferred to 31° at the intervals indicated.

HOURS AT 25°	♀♀			♂♂			PERCENT MORTALITY
	M	C.V.	N	M	C.V.	N	
6	—	—	—	—	—	—	100.00
12	0.95±0.04	10.41	14	1.64±0.06	17.20	9	57.49
18	0.94±0.02	11.62	23	1.47±0.05	14.97	8	42.60
24	0.93±0.02	9.21	14	1.55±0.03	10.70	12	51.75
30	1.03±0.03	17.90	25	1.33±0.04	26.49	29	50.00
36	0.90±0.02	10.72	19	1.20±0.04	23.70	24	20.38
42	0.92±0.02	14.40	33	1.23±0.06	36.85	28	43.51
48	1.06±0.04	28.81	33	1.04±0.04	29.50	32	39.80
54	0.91±0.02	12.31	22	0.82±0.01	13.57	41	41.70
60	0.93±0.01	11.83	31	0.88±0.03	20.42	20	53.72
66	0.83±0.01	15.00	47	0.81±0.01	15.45	43	16.67
72	0.92±0.03	16.91	14	0.77±0.01	8.82	17	42.55
78	0.74±0.01	8.43	21	0.72±0.01	8.74	25	14.82
84	0.77±0.01	12.82	45	0.66±0.01	11.50	40	21.30
90	0.71±0.01	9.93	50	0.65±0.01	10.80	40	16.68
25° control	0.80±0.01	15.08	132	0.74±0.01	11.21	129	—
31° control	1.06±0.04	25.87	31	1.63±0.05	22.41	25	—

are very high, especially when the larvae were first started in 25° and later transferred to 31°. These coupled with the small number of individuals measured made it hazardous to draw any definite conclusions as to the effect of high temperature on the wing development of the vestigial flies. However, as indicators of the time at which high temperature begins to exert its influence on the development of vestigial wings and when this influence ceases, the data do show certain things. In cases where the larvae started their development at 31° and then were transferred to 25° (table 4), it can be noticed that at about the sixtieth hour there is a decisive tendency for the vestigial wings to become longer. This point must be taken as the beginning of the temperature-effective period for 31°C. In the case of the opposite transfers (table 5) it is also easily seen that beginning with the seventy-eighth hour, the mean length of the vestigial wings becomes steadily shorter. This must mean that the temperature-effective period ends before the latter point is reached. On the basis of these observations we may fix the temperature-effective period for vestigial wings roughly at sixty to seventy-two hours in the larval period, making a total of approximately 12 hours. The data further show that at 31°, with the stock of vestigial flies used, there is a distinct sexual-dimorphism in the wing lengths such as observed by the earlier workers (ROBERTS, HARNLY and STANLEY).

In order to overcome some of the difficulties in variability and mortality encountered in the above experiments, a number of similar tests were made. In these later attempts, we used twice as many flies from a vestigial stock that had been inbred for nine generations. Another deviation in procedure from the earlier experiments was that after they had been isolated the larvae were allowed to develop for the first 24 hours at 25° without any disturbance, at the end of which, they were all transferred to 31°, except of course the 25° controls. From 31° at each of the succeeding 6-hour intervals, the larvae were again transferred to 25° to complete their development. No transfers in the opposite direction were made.

The reason for allowing all the larvae to pass the first 24 hours in 25° instead of 31° is primarily an attempt to reduce mortality of the larvae. Since the temperature-effective period is found to be approximately in the latter half of larval life, the elimination of the first day of high temperature treatment should not interfere with the result as far as the location of the critical period is concerned. At the same time, it may be of interest to see whether such elimination would affect the development of the vestigial wings. In order to test this point, two 31° controls were employed. In one of these, vestigial flies were raised throughout their larval stage at 31° (control 2, table 6), while in the other, the larvae were first exposed to 25° for one day like the rest of the cultures in the experiment and then were transferred to 31° (control 1, table 6).

Table 6 gives the results of one such experiment. It can be seen that

TABLE 6

Mean lengths in mm of vestigial wings of flies, kept at 25° for the first 24 hours of their larval period, raised at 31° and subsequently transferred to 25° again at the intervals indicated.

PERIOD AT 31°	♀♀			♂♂			PERCENT MORTALITY
	M	C.V.	N	M	C.V.	N	
24-30	0.81 ± 0.01	11.09	67	0.74 ± 0.01	12.12	56	38.5
24-36	0.81 ± 0.01	12.59	86	0.73 ± 0.01	15.92	88	13.0
24-42	0.79 ± 0.01	9.38	85	0.74 ± 0.01	9.37	69	23.0
24-48	0.81 ± 0.01	10.62	81	0.73 ± 0.01	8.87	76	21.5
24-54	0.80 ± 0.01	9.13	81	0.74 ± 0.01	9.36	73	23.0
24-60	0.85 ± 0.01	12.05	65	0.84 ± 0.01	6.02	62	36.5
24-66	1.02 ± 0.03	21.60	87	1.07 ± 0.04	29.03	89	12.0
24-72	1.07 ± 0.03	19.82	75	1.16 ± 0.04	21.85	64	30.5
24-78	1.01 ± 0.02	13.61	69	1.16 ± 0.04	24.42	57	37.0
24-84	0.92 ± 0.02	13.12	64	0.99 ± 0.03	25.30	78	29.0
24-90	0.99 ± 0.02	15.16	54	0.99 ± 0.04	26.50	65	15.5
25° cont.	0.79 ± 0.01	13.12	77	0.72 ± 0.01	8.94	98	12.5
31° cont. 1	1.03 ± 0.04	25.32	81	1.28 ± 0.04	26.10	84	17.5
31° cont. 2	1.73 ± 0.05	30.40	74	1.77 ± 0.05	21.01	32	47.0

beginning with the sixtieth hour of larval life, the lengths of the vestigial wings of the treated flies show a distinct increase, especially in the males. In the females the increase in lengths of wings is not very striking until the sixty-sixth hour interval. It is possible that by starting the larvae at 25° for the first day, the whole developmental process may be somewhat slowed down, as compared with those which were raised entirely at 31°, thereby causing a slight delay in the onset of the critical period. The slight drop in the mean wing lengths after the seventy-eighth hour interval may not be very significant. It should not be interpreted as the end point of the temperature-effective period; without data on the opposite transfers, such an end point cannot be determined with certainty.

Regarding the increase of wing length of the flies exposed to high temperature, one may note that the striking difference in wing lengths between the sexes is very much reduced if not entirely gone. This probably means that with inbred stock, more or less homogeneous in its make-up, the so-called sexual-dimorphism can be eliminated and in both sexes the development of vestigial wings then responds to high temperature equally well. However, when the first day of the larval period was not subjected to high temperature, neither sex of the vestigial flies could have wing lengths comparable to those whose whole larval period was subject to it. This is clearly shown in the two 31° controls (controls 1 and 2, table 6). So in order for the vestigial wings to reach the maximum length, the first 24 hours of the larvae must be treated with high temperature (in this case 31°). It follows therefore that high temperature at this particular period supplements the process involved in the enlargement of the wings. This supplementing process however could do nothing unless the larvae were continuously treated under high temperature during the critical period (60–72 hour interval).

The same point can be verified from yet another experiment. When only short intervals of 12 (60–72 hours) or 24 (48–72 hours) hours from the latter half of the larval period of the vestigial flies and covering the whole length of the temperature-effective period were subjected to 31° the average lengths of the wings of either sex could not be increased to more than 1.03 ± 0.01 mm (from unpublished data). In view of these facts, any assumption that the increase in size of the vestigial wings is due to high temperature treatment only at the critical period may be held as questionable.

THE FORMS OF THE WINGS OF THE TREATED VESTIGIAL FLIES

As we examined the wings of the treated vestigial flies at the time when they were measured, we noted that there was a great deal of variation in the forms of the wings. Greatest variation was found in flies longest exposed to 31° and in the 31° control. After having made all the necessary measurements, we fixed these flies in 95 percent alcohol. Wings were taken off from the flies very carefully with fine forceps under a binocular and dehydrated by passing through the diaphane solvent and finally mounted with diaphane. Drawings were made under the Edinger Drawing apparatus according to the same scale of enlargement.

The regular vestigial flies are not capable of flying but those raised in high temperature may have wings enlarged enough to fly almost as well as the wild type. However, their wings are comparatively thinner than those of the wild types and therefore are easily folded and injured. Balloon formation by the separation of the upper and the lower surfaces of the wings either partly or completely was of common occurrence especially when the flies were fixed in weaker alcohol.

In the untreated vestigial wings (figures 1 and 2), it can be shown that except the marginals, all the longitudinal veins of a typical Drosophila wing are present and easily identified under a binocular microscope. A short axillary vein accompanied by a humeral crossvein is also present. The first three longitudinal veins are branched from the main stem which is divided very distinctly by two transverse sutures. The fourth, fifth and the smaller sixth longitudinal veins are developed very irregularly. The anterior and posterior crossveins together with anal crossvein are entirely absent. Among the vestigial flies the wing forms are not at all uniform, but as a rule the wings look folded and thickened, apparently due to the contraction of the longitudinal veins.

For the sake of description the various wing forms produced by high temperature treatment are conveniently classified into five types as follows:

Type 1 (figures 3 and 4): These are similar to the untreated regular vestigial wings. However by careful study, one may note four differences: First, the edges are more or less invaginated at the point where the longitudinal veins end; second, the posterior and anal crossveins are usually present; third, the marginal hairs are developed in places along the costal and the first longitudinal veins; fourth, the wings are slightly longer and broader than the untreated vestigials.

Type 2 (figures 5 to 14 inclusive): In type 2 there are two subdivisions (a) and (b), one of which is obviously derived from the other.

(a) The wings are longitudinally well extended but slender and pointed at the distal end. The marginal cell is present as a narrow strip. The anterior and anal crossveins are well developed; and the second longitudinal vein is usually fully extended sometimes even bearing marginal hairs. The latter may also develop at the region in front of the distal end of the first longitudinal vein (figures 5, 6 and 7).

(b) The wings are similar to (a) except the distal end of the wing turns upward and is often enlarged and somewhat rounded (figures 8, 9, 10 and 11).

Type 3 (figures 12, 13 and 14): The wings are broader than those of type 2 and the distal end is somewhat blunter and broader than the proximal end. The posterior crossvein is usually present or at least shows a rudimentary development.

Type 4 (figures 15 to 20 inclusive): Wings in this case show the marked development of the submarginal and the second posterior cells to present the general appearance of a fork. The posterior crossvein is always present. Sometimes the submarginal cell, the second posterior and the third posterior cell are especially extended to form two or three projections at the distal portion of the wing.

Type 5 (figures 21, 22 and 23): In this type of wing, all the typical wild-type characteristics are present with the one exception that the margin may be cut or notched in one or several places. The wings are usually attached to the body of the fly in the same manner as that of the wild-type.

It must be noted that while type 1 and type 5 described above undoubtedly represent two extremes and conceivably the latter might have been resulted by an all round expansion of the former, one cannot be sure that the intermediate types (types 2, 3 and 4) belong to a progressive series from the narrow to the broad types of wings. On the contrary, they may have occurred quite at random. The similarity of these forms to strap, antlered and various other allelomorphic phenotypes has already been pointed out by earlier workers (STANLEY 1931, etc.).

It is interesting to note that in the vestigial wings of the untreated flies, all the longitudinal veins of the wild-types are present. The enlargement of these vestigial wings as a result of temperature treatment is accompanied by the extension of these longitudinal veins. The posterior crossvein appears only when the wing reaches a certain breadth and the finishing touches of the wing development are the marginal hairs.

Besides the types mentioned, there are several other peculiarities observed, such as wings which appear as a triangle or like a tongue and sometimes an extra crossvein parallel with the anterior crossvein may be developed. Perhaps the most remarkable fact of all that we have observed is that in a number of cases the right and left wings of the same individual were strikingly different (figures 26 to 29 inclusive).

The size and number of cells of vestigial wings after high temperature treatment

Vestigial flies obtained from the experiment just described have furnished material for this study. The flies were first cooked with 10 percent

KOH and washed through a series of alcohols before the wings were taken off and mounted in diaphane, which would make the wing very transparent. For cell counting, we have adopted the method of DOBZHANSKY (1929). The work was done under the Edinger apparatus. The submarginal cell of the wing was arbitrarily chosen as the marked area for hair counting because of the several advantages. 1 The particular cell is present almost in all cases; 2 it is clearly defined by the second and the third longitudinal veins; 3 this particular cell is very sensitive to temperature treatment; and 4 the hairs here are comparatively regularly arranged and evenly distributed. Two kinds of counts were made: one on the hairs of the whole area of the cell and the other on those within a standardized small area inside of the cell. Specimens from ten of the males for each of the differently treated vestigial cultures were taken at random and thus examined. The results of these counts are shown in table 7.

TABLE 7

The average number of hairs in the submarginal cell etc. of the vestigial wings of flies raised at high temperature during the larval stage in the various periods indicated.

PERIODS EXPOSED TO 31°C.	NO. HAIRS IN SUBM. CELL	LIMITS	NO. HAIRS IN STANDARD AREA	LIMITS
24-30	135	40-200	38.5	28-48
24-36	146	80-250	37.8	28-43
24-42	246	90-640	38.7	31-48
24-48	162	110-250	36.7	31-41
24-54	214	130-280	35.7	31-42
24-60	330	170-580	32.7	25-39
24-66	799	400-1120	35.1	31-39
24-72	771	480-1100	32.1	26-38
24-78	827	600-1170	32.3	28-35
24-84	828	570-1020	30.6	24-35
31° cont.	1094	940-1200	30.2	28-32
wild-type ♂♂	1723	1680-1760	20.7	19-22

It is evident from the results shown in the above table that the number of cells in the submarginal cell was increased with the increasing length of exposure to high temperature, while a somewhat reversed situation is seen in the case of cell counts within the standardized area. There is also a wide range of variability in both counts, but the range gradually diminished as the flies were exposed to high temperature for longer periods. Comparing with the wild-type males, one finds that the average number of cells per chosen area is more constant and both the number and size of the cells in the submarginal cell of the wing are larger in the wild-type than any of the treated vestigials. While the increase in size of cells in the wings of the treated vestigial flies may be due to a large extent to the stretching of the wings, the increase in number of cells must be the result of high temperature treatment. It is concluded therefore that the enlargement of the vestigial wings of the flies raised in high temperature is largely due to the increase in the number of cells.

Besides the experiment just described, we have also made a comparative study of the larval wing buds of (1) the vestigial flies (2) those under high temperature treatment and (3) the wild-type. As the results will be taken up in a separate paper, only a general statement will be made here. Beginning with the fifty-fourth hour of the larval period, the posterior part of the mesothoracic bud was seen to expand gradually. This change was more clearly seen in wild-type and in temperature-treated vestigials than in the untreated ones. From the sixtieth to seventy-second hour period the so-called wing bud begins to be formed from the posterior portion of the mesothoracic disc. The wing bud rudiments in wild-type and in treated vestigials are comparatively more pronounced than those of untreated vestigials. Comparing the time occupied by the temperature-effective period in the larval stage with that during which the changes of the imaginal discs occur, one may be led to conclude that high temperature at this critical period has an accelerating effect upon the growth of the mesothoracic bud and causes it to develop a larger wing bud in the vestigial flies.

DISCUSSION

It was ROBERTS (1918) who first discovered that there is a peculiar relation between high temperature and the development of the enlarged vestigial wings. He further showed that the critical period of temperature effect lies between the fertilization of the egg and the pupal stage. Among recent investigators along this line, HARNLY (1930, 1931 and 1933) and STANLEY (1928, 1931 and 1935) have done some very critical work. HARNLY (1930) found that there is a progressive increase in length of the vestigial wings with the progressive increase in temperature from 18° to 31°C. He later (1933) showed that the gradual enlargement of the vestigial wing under high temperature (30° 31° and 32°) follows a typical sigmoid growth curve and that the growth period extends from the sixtieth to eighty-fourth hour after the egg starts to develop.

Much detailed work has been done by STANLEY (1928 etc.) in locating the temperature effective period for such temperatures as 17°, 27°, 30° and 31° etc. Although both HARNLY and STANLEY started their experiments with only the age of eggs known, their data on the temperature-effective period for 31° are fairly comparable with what we found with the larva-isolation technique. It seems to us that the latter method of determining the critical period has at least two advantages. In the first place, we can get a more exact knowledge of the mortality rate of the larvae and, secondly, a more accurate determination of the time interval in the larval period as such. According to our data, this critical period begins roughly at the sixtieth and ends at the seventy-second hour of the larval period. This particular period approximates to the time when the imaginal discs for wings are about to be differentiated from the mesothoracic buds (CHEN 1929).

It is possible, as shown in our tests, that high temperature in the pupal period may have some effect upon the size of the vestigial wing, causing slight reduction in the size (table 3). We have also found that the temperature in the first 24 hours of the larval life tends to accelerate the enlargement of the wings provided the flies are also exposed to it during the temperature-effective period. By raising the larvae from the beginning of the larval period at 31°, the vestigial wings may reach an average length of 1.73 ± 0.05 mm for the females and 1.77 ± 0.05 mm for the males. These figures are quite comparable to those which STANLEY found for the same temperature (STANLEY 1935). But when the larva was allowed to develop at 25° for the first 24 hours and then raised through the rest of the larval period at 31°, there was a considerable reduction in the mean lengths of the vestigial wings in both males (1.28 ± 0.04) and females (1.03 ± 0.04). It is of interest to note that according to CHEN (1929) the mesothoracic disc, from which the wing anlage is derived, appears during the first 24 hours of larval life.

The fact that different temperatures give different growth curves (HARNLY 1933) and that they have also different effective periods (STANLEY 1935) indicate that the situation is by no means a simple one. The complex situation is further shown by the high degree of variability in wing form. It becomes necessary that the whole problem should be looked into from a somewhat different angle. A study therefore from the embryological point of view may help to clear up some of the confusion. GOLDSCHMIDT (1935) in connection with his study of genes and external characters has examined the development of a series of mutant types affecting the form of the wing in Drosophila, such as vestigial-notch, cut, beadex, Beadex-Jollos and vestigial. By comparing the changes of the imaginal discs of the wings in the various mutations mentioned above in the pupal stage (from 8 to 30 hours), he found that although the wings of the adults of the above differ from one another, they all start in the early pupal period from imaginal discs comparable in size and pattern. Later however degeneration (Erkrankung) of the epithelial tissues of the wing buds sets in first from the tip, next the hind border and then the front margin, causing the wings to be remodeled according to the type of genes affecting them. In the case of vestigial wing, the anlage according to GOLDSCHMIDT's findings is actually 2 to 3 times as large as the adult wing before the degeneration process sets in and makes it shrivel to produce the adult pattern.

GOLDSCHMIDT'S study of the series of mutations from vestigial-notch to vestigial led him to infer that in the case of vestigial, the imaginal disc of the wing is somewhat comparable in size and pattern to that of the wild-type, but the degeneration of the epithelial tissue of the anlage starts much earlier (in the larval stage) than the others, such as cut, beadex and Beadex-Jollos, thereby giving rise to a much smaller and abnormal type of wing. Accordingly, he is inclined to interpret the temperature effect on the development of the vestigial wing in a different way from what has been implied by the earlier workers. According to GOLDSCHMIDT, the increase in size of the vestigial wing as a result of temperature treatment during the development of the fly is possibly due to the delay of the onset of the degeneration process rather than the direct increase in the size of the anlage.

Our work with the cell counts of the vestigial wings of flies raised under high temperature shows clearly that there is a decided increase in the number of cells. Unless the temperature effect is to prevent more cells from becoming degenerated, which is unlikely (since high temperature in the pupal period tends to decrease rather than increase the size of wings in the vestigial flies), it must be taken to mean that it has an accelerating effect in the growth period. It is unfortunate that GOLDSCHMIDT has not examined the imaginal discs of the vestigial flies in the larval period, so it is not possible to say, from his evidence to what extent the anlage of the vestigial wing is comparable to the other types. Our as yet unfinished work in connection with the comparative study of the imaginal discs of the mesothorax and the wing of larvae from wild-type, vestigial and vestigial raised at high temperature tends to show that these discs are larger in the case of the treated than the untreated vestigials. Without direct evidence at hand, we may have to infer that the more rapidly growing buds of the treated vestigials are the result of a higher rate of cell division in the formative period before the anlage starts to degenerate. These facts may somewhat modify the point of view held by GOLDSCHMIDT and they tend to show that the ultimate size and pattern of the vestigial wings of flies raised in high temperature is the result of at least two interrelated factors: the enlargement of the anlage of the mesothorax and wing on one hand and the delay of the onset of degeneration process on the other. Perhaps the high degree of variation in size, form and period of temperature effectiveness may all be explained when these two factors are carefully analyzed.

SUMMARY

1. Newly formed puparia of vestigial flies in Drosophila were isolated in vials and treated with the two temperatures 31°C and 25°C. The vials were divided into two series; the puparia in one of the series were first exposed to 31°C and then transferred in each of the succeeding six-hour intervals to 25°C until the end of the pupal stage; those of the other series were used as the opposite transfers.

2. The wing lengths of the vestigial flies so treated were measured and compared with the controls to see if the temperature had any effect on the flies. The results of the experiments tend to show that high temperature (31°C) is not effective in lengthening the wings of the vestigial in the pupal period. Possibly it may cause a slight reduction in the mean lengths of the wings.

3. However, when in like manner, the same temperatures were applied to the newly hatched larvae of the vestigial flies, the data on subsequent wing measurements showed that the high temperature was very effective in enlarging the vestigial wings. The temperature effective period is located at an interval which begins at approximately the sixtieth and ends at the seventy-second hour of the larval period.

4. By raising the first-day larvae in 25°C and then subjecting them to high temperature treatment described above, it was shown that the mean wing lengths of the vestigials so treated were significantly shorter than the cases when the first twenty-four hours of the larvae were also spent in high temperature. The evidence thus indicates that in order to realize the maximum lengthening of the wing characteristic of the temperature 31°C, not only the so-called temperature effective period, but also the first twenty-four hours of the larval stage must be exposed to the temperature.

5. The forms of the wings of the vestigial flies that have been exposed to 31°C for the whole (or almost whole) larval period showed a high degree of variability. This variation seems to bear no definite relationship with the sex of the individual or the size of the fly. Furthermore, the right and left wing of the same individual may occasionally be strikingly different.

6. By making various hair-counts of the submarginal cell of the wings of the treated flies, it is possible to show that the gradual enlargement of the wings as a result of high temperature treatment is primarily due to the increase in number rather than size of the cells.

7. The results of the above observations lend themselves to the interpretation that the high temperature tends to cause an increase in size of the mesothoracic and later the wing buds by increasing the rate of growth and cell division in these bodies and that the temperature seems to be particularly effective at the time when these buds start to form in the larval period of the fly.

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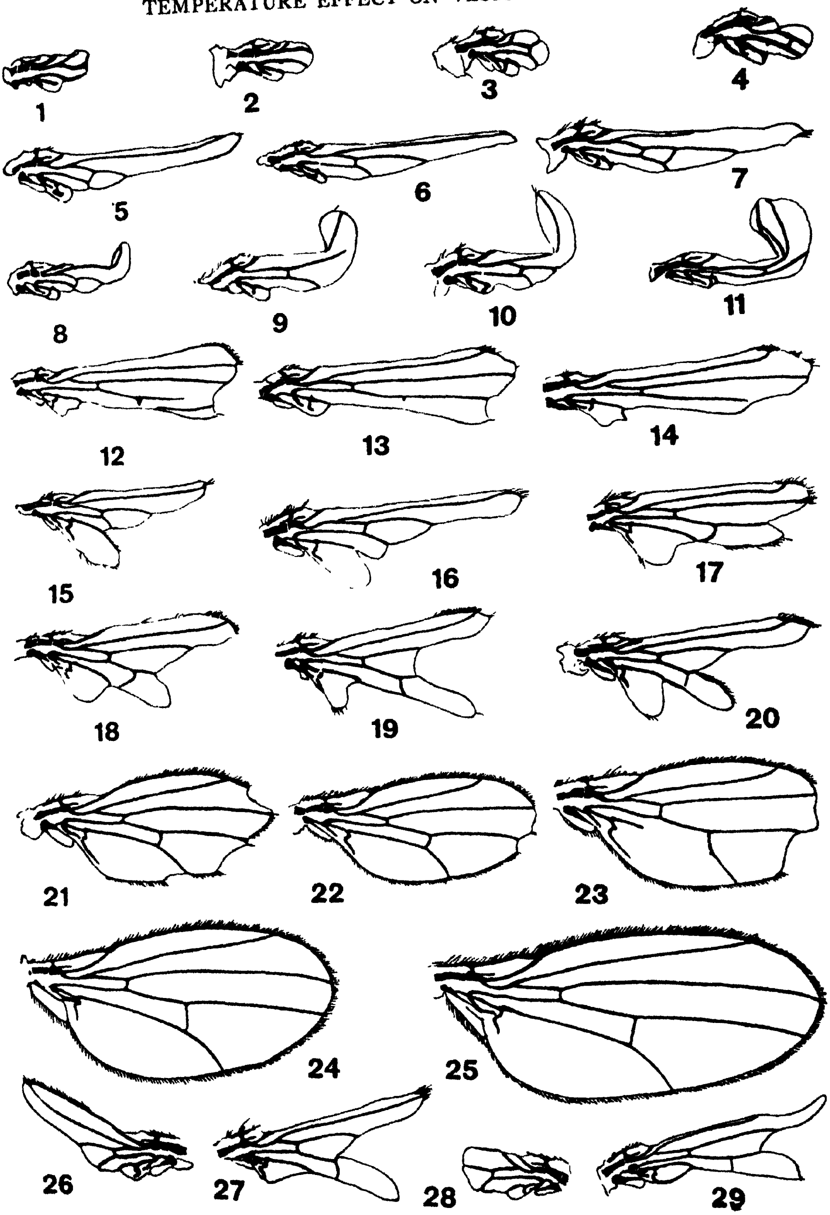

FIGURES 1, 2—Normal vestigial wings.
FIGURES 3, 4—Type 1 wing variations.
FIGURES 5–7—Type 2a wing variations.
FIGURES 8–11—Type 2b wing variations.
FIGURES 12–14—Type 3 wing variations.
FIGURES 15–20—Type 4 wing variations.
FIGURES 21–23—Type 5 wing variations.
FIGURES 24, 25—Wild type wings ♂ and ♀ respectively.
FIGURES 26, 27—Left and right wings in one individual.
FIGURES 28, 29—Left and right wings in another individual.

CYTOLOGIC STUDIES ON THE ABNORMAL DEVELOPMENT OF THE EGGS OF THE CLARET MUTANT TYPE OF DROSOPHILA SIMULANS

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INTRODUCTION

ALTHOUGH many instances of abnormal development of zygotes containing mutant genes have been described in the course of genetic studies on Drosophila, few attempts have been made to determine the mechanism of such development. Much of the material which has been studied has been poorly adapted to genetic analysis of this problem, since mutant genes usually produce developmental abnormalities so slight as to be difficult to identify, or so great as to produce complete inviability of the eggs containing such genes. The claret mutant gene of *Drosophila simulans* produces effects on the egg which are neither too great nor too small for study of this kind since some of these eggs develop into viable adults showing major abnormalities. For this reason genetic studies of the offspring of the claret mutant type have been completed, and have led to the suggestion of a probable mechanism for the production of the abnormalities found. A further study of this suggested mechanism may be made by the use of the cytologic method, basing such study on the genetic data at hand.

The writer wishes to express her thanks to Dr. ALFRED F. HUETTNER, under whose guidance the work was carried out, to Dr. RUTH B. HOWLAND for her assistance in the preparation of this report, and to Dr. ALFRED H. STURTEVANT for his kindness in reading and criticizing the manuscript.

REVIEW OF LITERATURE

In 1924, PLUNKETT found in a stock of *D. simulans* from Austin, Texas, a mutant fly with a claret eye color. Flies homozygous or heterozygous for this mutant gene were of good viability and normal in appearance, but homozygous females, on breeding, showed an additional abnormality. Although these females laid a normal number of eggs many of the eggs failed to develop, and those which did develop gave rise to adults only a few of which were normal. Females heterozygous for the mutant gene, and males both homozygous and heterozygous, produced the usual number of normal offspring. STURTEVANT and PLUNKETT, in 1926, located the claret mutant gene in the third linkage group, and found it to correspond to the claret mutant gene of *D. melanogaster*. The presence of the claret mutant gene in *D. melanogaster* is not associated, however, with developmental abnormalities. STURTEVANT, in 1929, published the results of his genetic studies on the offspring of females homozygous for the claret gene. He described the kinds of abnormalities found among the viable offspring, and the frequency of occurrence of these abnormalities. He confirmed the observation of PLUNKETT that from the females it was possible to obtain only a very small number of viable eggs.

In addition to the high frequency of inviability, STURTEVANT found a second abnormality among the viable offspring of the claret females. This was the occurrence of flies whose sex-linked characters were unlike those expected from the matings made. Of the males produced by a cross, 50 percent were such exceptions, the sex-linked characters being apparently determined by the paternal rather than the maternal X chromosome. Of the females, 6 percent showed exceptional sex-linked characters. Such anomalous flies have been found in other stocks where they have been shown to be produced by failure of the X chromosomes to disjoin during the maturation of the egg. This process, however, would give rise to exceptional males and females in equal numbers rather than to such disproportionate numbers as STURTEVANT found. Similar disproportions of exceptional males to females had been observed earlier in *D. melanogaster* by BRIDGES (1916), SAFIR (1920), MAVOR (1924) and ANDERSON (1924) and in *D. simulans* by STURTEVANT (1921). These investigators suggested that the inequality between males and females might have been produced by occasional failure of the X chromosomes to reach either pole of the maturation figure, or by elimination of both maternal X chromosomes during the first cleavage. Either of these processes would produce only exceptional males while non-disjunction would produce both exceptional males and females. The fact that the inequality of males and females in the claret stock of *D. simulans* is greater than that found in other stocks was not explained by STURTEVANT.

A third type of abnormality found by STURTEVANT was the occurrence of flies having small bristles and frequent imperfections of the last section of the fourth vein of the wing. These "diminished" flies were shown by chromosome studies to be haplo-IV in constitution. Since no fourth chromosome mutant was available, it was not possible to determine whether the missing chromosome was that of the paternal or the maternal set.

In addition to these abnormal types, STURTEVANT found flies which gave evidence of similar abnormalities involving, however, only part of the tissues of the fly. This type of abnormality involved the X chromosome in 3 percent of the offspring, resulting in the production of gynandromorphs. The fourth chromosome was involved in 4 percent of the offspring, resulting in the production of "diminished mosaics" which were haplo-IV in part of their tissues. The lack of a fourth chromosome mutant prevented further studies on the diminished mosaic flies.

From the complete studies of the sex-linked characters STURTEVANT found that two classes of gynandromorphs occurred among the offspring of the claret females. These classes may be described by a specific example. If a female, homozygous for the claret and yellow genes is crossed to a wild type male, the offspring are expected to be wild type females and yellow males. Exceptional yellow females and exceptional wild type males were also produced. The gynandromorphs produced by such a mating were of two classes. The first, including 90 percent of the gynandromorphs, consisted of wild type female tissue (phenotypically similar to the normal females produced by the mating) and wild type male tissue (similar to the exceptional males). The second class, including the remaining 10 percent, consisted of yellow female tissue (phenotypically similar to the exceptional females produced by the mating) and yellow male tissue, (similar to the normal males).

Additional observations which proved to be of importance in the formulation of a mechanical picture of the events causing the abnormalities of these eggs may be mentioned. In both the sex and fourth chromosome mosaics the abnormal tissues most often comprised ½, ¼, or ⅛ of the fly. More rarely fractions other than these were observed. Further, an abnormality of one linkage group in the whole or part of a fly was not necessarily accompanied by an abnormality of another linkage group. No major abnormality involving the second or third linkage groups was observed.

From these results, STURTEVANT set up the following hypothesis to account for the abnormalities found. Elimination of chromosomes may occur during the maturation divisions of the egg or during the early cleavages. Elimination of an X chromosome during maturation produces a female pronucleus deficient in its X chromosome, and fusion of this with a male pronucleus produces an XO male or an inviable YO zygote. (Exceptional females may be produced by a second process, non-disjunction). Elimination of a fourth chromosome during maturation produces a fly of haplo-IV constitution. If an X or a fourth chromosome is eliminated from one of the nuclei formed during the first cleavage, a fly is produced which is deficient for this chromosome in approximately half of its tissue. Elimination during later stages of cleavage produces flies which are abnormal in varying amounts, depending on the time of elimination of the chromosome. Elimination of a second or a third chromosome during maturation or the cleavage divisions produces an inviable zygote.

To account for the peculiar classes of gynandromorphs found, a further assumption was necessary. As indicated above 91 percent of the gynandromorphs had normal female and exceptional male tissue. If, according to

STURTEVANT'S hypothesis, the exceptional male tissues are produced by the elimination of an X chromosome during cleavage, it must, in these cases, be the maternal X which is eliminated. This process produces a fly in which the male parts are XO in constitution, the remaining X chromosome being that of the paternal set. The assumption may also explain the second class of gynandromorphs, which may be produced by the elimination of a maternal X during cleavage, if such a chromosome is eliminated in an egg which began its development as an exceptional (XXY) female. The female tissue then remains exceptional while the male tissue, having suffered the loss of one of the X chromosomes, becomes similar genetically to the normal males expected from the same mating.

In other reported cases of the occurrence of gynandromorphs, no evidence of the selective elimination of maternal chromosomes has been found. Apparent evidence of such elimination was reported by BRIDGES (1925) in Minute-n *D. melanogaster*. But here elimination was of this type because the mutant gene caused elimination of the chromosome which carried it. If it had been carried by the paternal chromosome, that chromosome would have been eliminated (STERN, 1927).

In the cytologic study of the eggs of homozygous claret females STURTEVANT'S hypothesis has been a valuable working guide, indicating as it does, the probable stages in development when abnormalities are likely to occur. However, if an attempt is made to apply the hypothesis as a mechanical picture of the possible cytologic events, the explanation becomes less simple than it appears at first. To picture a mechanism which will permit the loss, during cleavage, of chromosomes of the maternal set, and rarely or never, those of the paternal set, is difficult. For, with the completion of the first cleavage, the two sets of chromosomes lie together in close association. To drop those of one set selectively would require a peculiar and complex mechanism.

A new hypothesis for the production of gynandromorphs has therefore been set up on the basis of the cytologic data. This hypothesis, to be presented below, fits the genetic data, and in addition lends itself to the postulation of a simple cytologic mechanism for producing the abnormalities. Further, an attempt will be made to establish the validity of STURTEVANT'S suggestions that chromosomes may be excluded from the female pronucleus as a result of the peculiarities of the first maturation division, and that this process, when it involves the second or third chromosomes results in failure of the egg to develop.

MATERIAL AND METHODS

To secure the eggs for study, a stock was used from which both normal and abnormal material could be selected. Since homozygous claret females produce few viable eggs, the stock was maintained by mating heterozygous females with males homozygous for the mutant. For the study of the abnormal eggs, matings were made between homozygous claret females and heterozygous males. For control material, to show the normal course of development of *simulans* eggs, heterozygous females were mated to homozygous claret males. The stocks used were also homozygous for the third chromosome recessive, scarlet. This mutant gene had no appreciable effect on the viability of either the homozygous or heterozygous claret flies.

Flies were segregated as they emerged and fed on banana-agar mixture for five days before mating. Females were starved for several hours before collection of eggs was to begin, and permitted to mate. The wings of the females were cut off under ether to permit the posterior end of the abdomen to be observed during laying. For the collection of eggs such flies were placed in vials containing a glass slide on which was placed a piece of dark blotting paper moistened with fermented banana and yeast. During the period of laying these flies were kept at a temperature of 25.5 to 26.5° C.

To obtain abnormal and control eggs of comparable ages it was necessary to determine for each egg used the time interval between fertilization and fixation. In flies from which the wings have been removed, it is possible to see, after an egg has been laid, several protrusions of the uterus before the laying of the next egg. In the first few of these protrusions, the uterus appears yellow, translucent, and pointed at the posterior end. After several such protrusions the uterus, during one of them, becomes wider, and after another slight movement, becomes opaque, blunt at the end, and presents a shiny white tip. This tip is the posterior end of the egg, which has reached the end of the uterus. The anterior end of the egg is now in position to receive sperm. Fertilization was therefore assumed to occur when the white tip appeared at the end of the uterus, and timing of the eggs was begun at that moment. Slides made from control eggs timed in this manner showed similar stages of development when allowed to develop for similar lengths of time, justifying the timing procedure used.

The position of each egg on the blotting paper and the time of fertilization was recorded. After the desired time interval had elapsed, the slide was removed from the vial. The eggs were transferred to Kahle's formol-alcohol-acetic fixative and punctured at once with a sharpened steel needle (HUETTNER, 1923). To avoid distortion of the maturation figure which is found on the dorsal surface of the egg near the anterior end, eggs were punctured on the ventral surface, posteriorly. The puncture, when successful, permitted only a minute globule or a thin strand of oöplasm to escape.

Eggs were stained with erythrosin in 85 percent alcohol to facilitate orientation in paraffin under the binocular microscope. Sections were cut sagittally, 7μ in thickness and stained with Heidenhain's iron haematoxylin.

DESCRIPTION OF CYTOLOGIC PREPARATIONS

1. Control stock—development of normal eggs of D. simulans

The development of the normal control eggs follows closely that described by HUETTNER (1924) for *D. melanogaster*. With one exception the chromosomes of the two species are similar. The Y chromosome of *D. melanogaster* consists of one long and one short arm. The short arm is absent in the Y chromosome of *D. simulans*. The earliest fertilized eggs obtained were in the anaphase of the first maturation division. This figure lies, as in *D. melanogaster*, at the periphery of the egg, in a small, yolk free, cytoplasmic island on the dorsal surface near the bases of the filaments of the chorion. In eggs fixed 5 minutes after fertilization the chromosomes lie at the poles of the first maturation spindle (figure 1). No reorganized nuclei are seen at the completion of this period, the chromosomes lining up immediately on the spindle for the second maturation division. Stages of this division are found in eggs fixed between 5 and 10 minutes after fertilization (figure 2). Eggs which have developed for 11 minutes (figure 4), show the four reorganizing polar nuclei, three of which, as is true in *D. melanogaster*, remain in the cytoplasm at the periphery of the egg. One of the four nuclei lies nearer the center of the egg, where the sperm, not shown in this figure, is undergoing reorganization.

Preparations of eggs fixed during the next few minutes do not permit of identification of the reorganized egg nuclei which at this time stain poorly, and are again visible as clear vesicles only in preparations of eggs which have been permitted to develop for 15 minutes. Eggs prepared during the next 5 minutes show these four nuclei, each containing a haploid set of chromosomes. One of these vesicles, the female pronucleus, lies near the center of the egg, in the protoplasmic island containing the male pronucleus, wherein the haploid set of chromosomes is also visible. Material fixed from 20 to 23 minutes after fertilization shows the polar chromosomes lying free in the peripheral cytoplasm in three haploid groups, the nuclear membranes having disappeared. At this stage, two spindles are seen in association with the chromosomes of the male and female pronuclei. Figure 4 shows a portion of such a preparation, in which the three haploid groups of polar chromosomes may be seen. The cleavage figure present near the center of the egg is not shown. During later cleavages the two spindles characteristic of the first cleavage are no longer to be seen, the maternal and paternal chromosomes being closely associated on a single spindle. Such late cleavages further indicate the persistence of the polar chromosomes or nuclei in the peripheral cytoplasm.

2. *Homozygous claret stock—development of abnormal eggs of D. simulans*

a. First maturation division. As in the control preparations, the abnormal eggs immediately after fertilization are in the anaphase of the first maturation division. The figures at this stage are distinctly abnormal, showing one general type of abnormality; namely, separation of the chromosomes at either end of an abnormally wide maturation spindle. Figure 5 shows such an abnormal figure. The chromosomes are greatly separated at one pole, less so at the other. Other preparations show modifications of this type of abnormality, the extreme being a separation of the chromosomes into several widely scattered groups as seen in figure 12. The spindles in these figures are correspondingly distorted.

b. Second maturation division. The abnormal eggs again differ from the control eggs at the time of the second maturation division. In the control stock, stages of the second maturation division are found in eggs prepared from 6 to 10 minutes after fertilization. Those of the abnormal stock of comparable age seldom show a second maturation division in progress, the chromosomes lying as they did at the end of the first maturation division. In some preparations the double nature of these chromosomes is already evident. During the next 5 minutes of development preparations show no evidence of the polar chromosomes, which lose their staining capacity as do those of the control preparations at the conclusion of the period of the second maturation division.

Fixation 15 minutes after fertilization discloses the presence of the vesicular nuclei formed by the reorganization of the chromosomes at the end of the maturation divisions. In contrast to similar preparations of control eggs, where four such nuclei are seen, the abnormal egg shows the presence of four to twelve reorganized nuclei.

c. Cleavage. In material fixed during the following period (20 to 30 minutes after fertilization) the chromosomes within the polar nuclei and pronuclei are visible. The chromosomes are long and thin, twisting about each other, thus making identification of the fourth chromosomes, the smallest of the set, impossible. The other chromosomes, however, are readily recognized. Where more than four of these egg nuclei are present, each contains less than the haploid number of chromosomes. The extreme of this condition is seen in those preparations where twelve nuclei are present, each containing but a single chromosome (figure 6).

The pronuclei in preparations of this age again show differences from those of the control preparations, where each of the pronuclei, lying side by side, showed the presence of its own spindle. In the abnormal stock the male pronucleus occupies its normal position and contains a normal spindle. The female pronucleus may be abnormal in position and in spindle formation. One or several of the nuclei produced by the maturation divisions may lie in association with the male pronucleus, but in many cases all these egg nuclei lie at the periphery of the egg so that no one of them may be identified as a female pronucleus. Spindles are rarely seen in association with any of these egg nuclei. When the total number of such nuclei is twelve, no spindle is ever seen in association with the female pronucleus, even when this is normal in position. Many preparations with fewer nuclei also lack the spindle of the female pronucleus. Figures 7 and 8 show two unusual preparations in which spindles are present in both pronuclei. In each of these, the female pronucleus contains both the second and third chromosomes, figure 7 indicating further a lack of an X chromosome and figure 8 the presence of an extra X chromosome. In no preparation was the female spindle present when the female pronucleus lacked a second or a third chromosome.

The majority of the preparations at hand show no cleavage figure. Eggs fixed during the next few minutes may still show the presence of the sperm spindle, but this is lost in older preparations, the male pronucleus appearing again as an interkinetic, lightly staining vesicle. Still later preparations, where second and third cleavages figures are expected, show instead the picture of nuclear degeneration, the pronuclei and polar nuclei being irregular in outline and lightly stained. An increased number of polar nuclei is visible at this time, the number of nuclei approximately doubling as the period for each unrealized cleavage passes. Spindles are not seen in association with this nuclear increase.

Those preparations which show evidence of normal cleavages following the first, still show the presence of polar chromosomes whose nuclear membranes have disappeared. These polar chromosomes lie scattered throughout the cytoplasm between the periphery of the egg and the centrally located protoplasmic islands containing the cleavage nuclei (figure 9). As in the more normal preparations, the number of these chromosomes increases at each cleavage.

d. Unfertilized eggs. It is important to determine the time of development at which the first sign of abnormality appears in the eggs of the claret females. Preparations were therefore made of eggs dissected from females before fertilization or laying. Figure 10 shows the earliest of these, in which the prophase of the first maturation division is seen. Figure 11 shows a later prophase figure. Both these preparations show no sign of the abnormality to follow. Figure 12 shows the first visible sign of the distortion of the maturation figure, groups of chromosomes being widely separated in the cytoplasm rather than in the single compact group characteristic of the control preparations at this stage of development.

THEORETICAL DISCUSSION

In the discussion to follow, consideration of the fourth chromosome will be omitted. This chromosome is small and is identified in normal eggs by its position in close association with the other chromosomes. In the abnormal preparations where the chromosomes are scattered, this relation is lost, and it becomes impossible to differentiate the small chromosome from nearby yolk granules of the same size.

The first abnormality which appears in the development of the eggs of the homozygous claret females is the distortion of the first maturation spindle and the separation of the chromosomes at its poles. The subsequent abnormalites of development may be explained as results of this initial abnormality. To carry out this explanation each of the abnormalites will be reviewed in the light of those which took place in the preceding stages of development.

The picture presented at the time of the second maturation division may be considered to consist of two distinct abnormalities. The first of these is the failure of the second maturation to occur in orderly fashion in most eggs. Spindles for the separation of the chromosomes at this time are rarely seen. During this period the chromosomes lie as they did at the conclusion of the first maturation division, three long chromosomes being visible at each pole of the spindle. The small fourth chromosome is difficult to identify. These six chromosomes disappear during the next few minutes, and on their reappearance are found to have increased to twelve. Thus the chromosomes have doubled during the period of the second maturation division, but this division was an abnormal one, unaccompanied by any spindle.

The second abnormality of this period becomes evident at the conclusion of the second maturation period, and concerns the formation of an abnormal number of polar nuclei, each containing fewer chromosomes than the haploid set. The chromosomes at one end or at both ends of the distorted first maturation spindle are often widely separated from each other. No immediate effect of this is visible since no nuclear reorganization follows this division. However, when nuclear reorganization does occur, at the completion of the period of the second maturation division, it is apparent that the widely separated chromosomes have not been able to enter in normal fashion into the organization of these nuclei. For, instead of forming a compact nucleus containing all the chromosomes at one pole of the spindle, these chromosomes, scattered widely from each other, form instead, separate nuclei, each containing only a single chromosome. If each of the four chromosomes in each of four haploid sets found at the end of the two maturation divisions were thus to form its own nucleus, sixteen nuclei would be found. Such is not the case, however, the largest number at this time being twelve. The small fourth chromosome does not form its own nucleus, either remaining with one of the others or, if separated, failing to form a membrane about itself. This point could not be determined because of the difficulty of following the fate of the fourth chromosome.

The phenomenon of the formation of nuclei by single chromosomes has been called "idiomerie" by HAECKER and other workers, and occurs in both normal and experimentally treated tissues. POLITZER (1934) has reviewed the early work in this field. STRASBURGER (1880) and HAECKER (1895) described the formation, during normal mitosis of independent vesicles formed by each independent chromosome, these blending to form the typical single interkinetic nucleus. SCHILLER (1909) and TOBIAS (1914) were able to accentuate this slight degree of normal idiomerie by treatment of the eggs of Cyclops at high temperature. In all these cases the several vesicles fused finally to produce a normal interkinetic nucleus. ALBERTI and POLITZER (1934) so accentuated idiomerie by treatment with X-rays that the independent vesicles failed to fuse. It is this type of idiomerie that is found to occur in the untreated eggs of the claret mutant females under consideration.

The varying number of polar nuclei found in the abnormal eggs ready for first cleavage is the result of varying degrees of abnormality of the first maturation division. Complete separation of all the chromosomes at this time, with the exception of the fourth chromosome which apparently does not act independently, would produce twelve nuclei. Other variations in the number of these nuclei may be produced by partial scattering of the chromosomes of the first maturation division, separating two chromosomes from a third, or separating only those at one pole as in figure 5.

The next abnormality to be seen is the failure of cleavage in the greater number of eggs studied at this stage. Cleavage fails in several types of eggs. Such eggs include those in which twelve nuclei are present after the maturation divisions, none of these nuclei ever showing the formation of a maternal cleavage spindle. Also, eggs in which these nuclei are more nearly normal in number often fail to cleave. In the rare cases in which cleavage does occur, the female pronucleus is always found to contain at least the second and third chromosomes, in accordance with the genetic data presented by STURTEVANT.

We may further compare those chromosome groupings in the female pronucleus which permit of normal cleavage with others which do not. Figures 7 and 8 show two eggs in which the first cleavage is in progress, each of these eggs containing the diploid set of second and third chromosomes. Figure 7 indicates further a lack of an X chromosome in the female pronucleus and figure 8 the presence of an extra X chromosome, lying in its vesicle just outside the female pronucleus. Despite the X chromosome irregularities, cleavage is in progress. Figure 6, on the other hand, shows an egg which has failed to cleave. Surrounding the normal male pronucleus are three of the twelve nuclei produced by the maturation divisions, these containing one of the second, third and X chromosomes. These three chromosomes, when associated with the male pronucleus in other eggs, were sufficient to permit of normal cleavage. Here, where the necessary chromosomes lie in separate nuclei, cleavage has not taken place. It is thus apparent that in order for normal cleavage to occur, the second and third chromosomes must reach the male pronucleus and that they must do so enclosed within a single nucleus. If they do not lie in a single nuclear membrane, no spindle is formed for their division, and cleavage fails. Figure 8 illustrates this further. Here a single female pronucleus containing the second, third and X chromosomes is functioning in cleavage. But an extra nucleus (one formed by the maturation divisions), lying above the female pronucleus and containing a single X chromosome, has formed no spindle and has been unable to enter the cleavage figure up to this time.

The first abnormality observed, that of the first maturation division, is thus again responsible for the abnormality found at this time. Moreover, this early abnormality has at last achieved the result of stopping any further development of the egg, by causing, in most cases, the separation of the second and third chromosomes. Cleavage could take place only when these chromosomes remained sufficiently close at the end of the maturation division to become incorporated into a single nucleus. Cleavage could include the other chromosomes only when these remained sufficiently close to the second and third to be included with them in a single nucleus. Further, cleavage failure in these cases is seen to be characterized by the failure of spindle formation in the female pronucleus, for at this stage no spindle is ever formed by a female pronucleus deficient in the second or third chromosomes.

This observation lends itself to orderly interpretation if we assume that an interaction between the second and third chromosomes, or portions of them, is necessary in order that a spindle be produced. Re-examination of the abnormal maturation figures shows the accordance of these with the assumption made. For here, at the first maturation division, the second and third chromosomes are often separated from each other, relatively few eggs developing normally at this time. These abnormal eggs, if the assumption is correct, should now fail to form a spindle for the second maturation division. This is found to be the case.

The assumption that no spindle is ever formed unless the second and third chromosomes are in close proximity leads to the corollary that the presence of a spindle at any time during development must have been preceded immediately by a close proximity of these chromosomes. However distorted the spindle may be, then, the preceding prophase must have been normal, containing in a single nucleus all the chromosomes to appear on the spindle at the succeeding metaphase. This test of the validity of the assumption made must be applied to the first maturation division, the last appearance of spindle formation in the eggs that are completely abnormal. This spindle, if the assumption is valid, must be preceded by a prophase in which all the chromosomes lie in a single nucleus, surrounded by a nuclear membrane. This is found to be true. No preparations have been found in which the nucleus during the prophase of the first maturation division was abnormal. In every case the germinal vesicle was single, containing within it all the chromosomes. Figure 10 shows the single germinal vesicle of an egg before laying. Figure 11 shows the nucleus of a later stage of development of an unlaid egg, in which the nuclear membrane has just broken down. Again the chromosomes are found in a single compact group. Figure 12 shows the anaphase of the first maturation division. The spindle is completely formed, and for the first time the chromosomes are seen to be widely separated from each other in the cytoplasm. In such an egg, where the second and third chromosomes no longer lie together, no orderly mitotic figures can again be formed and the egg can never develop into an adult. Thus at no stage of development have figures been found which will invalidate the assumption that the second and third chromosomes must be in close proximity for spindle formation to take place.

STURTEVANT'S hypothesis for the production of gynandromorphs and fourth chromosome mosaics suggests that a chromosome may be dropped from one pole of the cleavage figure. To determine whether this process ever occurs or not is difficult, since the formation of gynandromorphs is rare. The fact that no evidence of the elimination of chromosomes has been found cytologically is thus no proof that this process never occurs.

The cytologic preparations studied, however, indicate that gynandromorphs may be formed by a process simpler than that which STURTEVANT suggests; namely, by the addition of a chromosome to a deficient nucleus. This new hypothesis accounts as well as does STURTEVANT'S for the two types of gynandromorphs which he found. Figure 7 shows the possibility of formation of the first class of gynandromorph, that having exceptional male and normal female tissue. The figure shows a preparation in which first cleavage is in progress. The diploid set of chromosomes lacks a single X chromosome, which was excluded from the female pronucleus by the separation of the chromosomes at the first maturation division. Near the upper pole of the figure, beyond the centriole, a single X chromosome is seen, lying free in the cytoplasm. This chromosome must be one that had been eliminated during a previous division, since it does not lie in a position indicating its elimination during the division in progress. Since this is the first cleavage the extra chromosome can only have been eliminated during the maturation divisions of the egg. It is therefore derived from one of the abnormal polar nuclei formed at that time. Further, this chromosome lies in what may become the path of the upper set of cleavage chromosomes when the division progresses. If, during this or later cleavages, the chromosomes at one pole of the cleavage figure lie close to the region occupied by the extra chromosome, the latter may become incorporated within that nearby cleavage nucleus. That one cleavage nucleus would then be enriched by this chromosome to contain the diploid set of X chromosomes, the remaining nuclei still being deficient for this chromosome and containing only the single X chromosome present before cleavage began. The deficient nucleus would thus lack a maternal X chromosome, missing since the formation of the female pronucleus. A gynandromorph would result in which the female parts, derived from the enriched nucleus are normal, and the male parts carrying a single X chromosome derived from the paternal set, are exceptional. This is the type of gynandromorph which was found by STURTEVANT to make up over 90 percent of those studied.

The egg pictured in figure 8 shows the possibility of formation of the second class of gynandromorphs. Again first cleavage is in progress but in this case the female pronucleus is complete. Above the complete maternal group of chromosomes is a vesicle containing an extra X chromosome which is not at this time entering into the cleavage. During cleavage the nuclear membrane surrounding this chromosome breaks down, setting the chromosome free in the cytoplasm as are those shown in figure 7. The extra chromosome may then pass to one pole of the cleavage figure, there to enrich one of the cleavage nuclei. If the egg pictured began its development as a normal male, the enriched nucleus now becomes XXY in chromosomal constitution, the other nucleus remaining XY as before the cleavage. A gynandromorph is thus produced in which the female tissues, derived from the enriched nucleus are exceptional female in constitution, and male tissues, derived from the unenriched XY nucleus are normal male. Genetically the result is the same as that obtained by STURTEVANT'S method, in which he suggests the formation of this type of gynandromorph by the elimination of an X chromosome from an egg which began its development as an exceptional female.

That chromosomes may be picked up during later cleavages is indicated by figure 9, in which, during the third cleavage, polar chromosomes are still seen to persist in the cytoplasm. Gradual degeneration of these chromosomes accounts for the fact that few gynandromorphs are found in which very small mosaic patches occur.

Such chromosome additions at later cleavages must be discussed more fully than the simpler first cleavage additions which produce gynandromorphs that are half male and half female. The addition of chromosomes at later cleavages produces gynandromorphs that are more than half female or less than half female. Those that are more than half female are produced when a chromosome is added at a later cleavage to an egg that has already undergone addition at first cleavage. Those that are less than half female are produced by additions of chromosomes at later cleavages to eggs that have undergone no previous additions. STURTEVANT (1929) found that 45 percent of all the gynandromorphs are more than half female while only 15 percent are less than half female. To fit the addition scheme to these facts it is necessary to show that chromosome additions occur so frequently at first cleavage, and so frequently again at later cleavages, that 45 percent of the eggs are likely to suffer at least two successive additions. Estimates of the number of eggs affected by abnormality at each cleavage, if the elimination hypothesis is used as the basis for such estimates, do not lend themselves to the support of the addition scheme, as indicated by Dr. STURTEVANT in a personal communication to the writer. However, estimates based on the elimination hypothesis may not be applied directly to the addition scheme.

The error in such a direct application may be illustrated as follows. Cleavage abnormality, if that abnormality is elimination, produces male tissue. The extent of male tissue in the adult indicates, therefore, the developmental stage at which this specific abnormality occurred. Each gynandromorph which is one half or more than one half male has thus developed from an egg in which elimination occurred at first cleavage, or successively at first and later cleavages. This group of gynandromorphs includes 55 percent of the total, and indicates the frequency with which first cleavage elimination occurs. Addition of chromosomes, on the other hand, produces female tissue, and the extent of female tissue in the adult indicates the developmental stage at which this particular abnormality occurred. Here, each gynandromorph that is one half or more than one half female has developed from an egg in which addition of chromosomes occurred at first cleavage, or successively at first and at later cleavages. This group of gynandromorphs includes 85 percent of the total, and indicates the frequency with which first cleavage addition occurs. Such estimates can thus be made only when a specific mechanism is under consideration, and the estimates based on one mechanism do not apply when the other mechanism is considered.

Similarly, second cleavage elimination is characterized by gynandromorphs that are one quarter, three quarters or more than three quarters male. These total 35 percent. Second cleavage addition, however, is characterized by gynandromorphs that are one quarter, three quarters or more than three quarters female. These total approximately 53 percent.

Thus the elimination scheme implies that 55 percent of the eggs become abnormal at first cleavage and 35 percent at second cleavage. On this basis it is to be expected that 19 percent (55 percent × 35 percent) of the gynandromorphs will bear evidence of successive elimination of chromosomes at both first and second cleavage. STURTEVANT'S counts of such gynandromorphs, characterized by the elimination scheme as being more than one half male, number 15 percent of the total. The addition scheme, however, leads to the expectation of 45 percent (85 percent × 53 percent) of gynandromorphs bearing evidence of successive addition of chromosomes at first and second cleavage. Counts of such gynandromorphs, characterized by the addition scheme as being more than one half female, number 45 percent of the total. The actual percentages here are not significant, because of the necessarily rough estimates of each class of gynandromorph made by STURTEVANT. They indicate, however, that either scheme may be used to explain the relatively large amount of female tissue found in the gynandromorphs.

The addition scheme may thus explain the formation of gynandromorphs and account for two peculiarities shown by them, namely, the absence of the maternal chromosome in over 90 percent of the gynandromorphs, and the fact that generally half or less than half of tissues of each of these gynandromorphs lack this chromosome. While the addition scheme presents no advantage over the elimination scheme in explaining the genetic data, it presents the advantage of a mechanical basis for the fact that it is always a maternal chromosome, derived from the egg, that is lacking in the deficient tissue. As the direct result of the abnormality of the first maturation division, this chromosome failed to be included in the female pronucleus and was thus lacking before cleavage began. When no additional chromosome is picked up by one of the cleavage nuclei the entire fly is deficient for this chromosome. When one of the nuclei produced by the first cleavage is enriched, only half of the tissue remains deficient for the maternal chromosome. A similar process during later cleavages will produce varying amounts of male and female tissue. The gradual degeneration of the extra polar chromosomes will reduce the frequency of this process in older eggs, making less likely the formation of gynandromorphs with small patches of mosaic tissue.

SUMMARY

1. Cytologic preparations of the eggs of the claret mutant type of *Drosophila simulans* are described.

2. The initial abnormality in these eggs is the separation of the chromosomes at each pole of the distorted spindle of the first maturation division.

3. The second abnormality is the failure of the second maturation

division in most eggs, and the reorganization of the chromosome groups at the end of this period, into an abnormally large number of nuclei, each containing an abnormally small number of chromosomes.

4. The third abnormality is the failure of cleavage in most eggs. This is correlated with the failure of a maternal spindle to appear in the absence of the second or third chromosomes from the female pronucleus.

5. These events are explained in the light of the abnormality of the first maturation division.

6. A new hypothesis is presented to account for the formation of gynandromorphs in this stock. The hypothesis is based on the cytologic data presented.

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EXPLANATION OF FIGURES

The figures were drawn with a Leitz 10X ocular and a Leitz oil immersion objective, N A 1.30, at table level with the aid of an Abbe camera lucida. A magnification of about 2540X was obtained. The figures have been reduced to one half in reproduction.

FIGURE 1.—Control stock. First maturation division. Five minutes after fertilization.

FIGURE 2.—Control stock. Second maturation division. The chromosomes at the extreme left will form the female pronucleus. Ten minutes after fertilization.

FIGURE 3.—Control stock. Reorganization of the four egg nuclei following the second maturation division. The nucleus at the left will form the female pronucleus. Eleven minutes after fertilization.

FIGURE 4.—Control stock. Three polar nuclei remaining at the periphery of the egg during cleavage. Twenty minutes after fertilization.

FIGURE 5.—Abnormal stock. First maturation division. The spindle is distorted and the chromosomes scattered at its poles. Five minutes after fertilization.

FIGURE 6.—Abnormal stock. Period of first cleavage. Twenty minutes after fertilization.

(a) Nine reorganized egg nuclei remaining at the periphery. Each contains a single chromosome. In the preparation these appeared in seven consecutive microscopic sections.

(b) Male pronucleus and three reorganized egg nuclei, that at the left containing the X chromosome, those at the right the second and third chromosomes. Figure reconstructed from two consecutive microscopic sections, one containing the male pronucleus and the autosome at the lower right, the other containing the X chromosome and the autosome at the upper right.

(c) Diagrammatic reconstruction of the above. The group of nuclei at the periphery at the left are the nine nuclei shown in (a), the central group the pronuclei shown in (b).

FIGURE 7.—Abnormal stock. First cleavage. Above the upper centriole is an extra X chromosome lying free in the cytoplasm. Twenty minutes after fertilization.

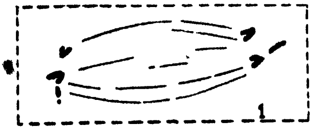
FIGURE 8.—Abnormal stock. First cleavage. The figure shows the paternal chromosomes at the left and the maternal at the right, these constituting a full haploid set. An extra vesicle containing a single chromosome lies above the maternal group. It has failed to form a spindle. Twenty-three minutes after fertilization.

FIGURE 9.—Abnormal stock. One of four nuclei of the prophase of the third cleavage. Two polar chromosomes are seen near it, lying free in the cytoplasm. Thirty-six minutes after fertilization.

FIGURE 10.—Abnormal stock. Prophase of the first maturation division. Single germinal vesicle. Unlaid, unfertilized egg.

FIGURE 11.—Abnormal stock. Shows a later stage than the previous figure. The nuclear membrane has broken down, the nucleus now lying at the dorsal surface of the egg. Unlaid, unfertilized egg.

FIGURE 12.—Abnormal stock. Shows a later stage than the preceding figure. The spindle is distorted, the chromosomes scattered into three widely separated groups. Unlaid, unfertilized egg.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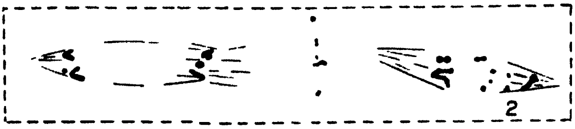
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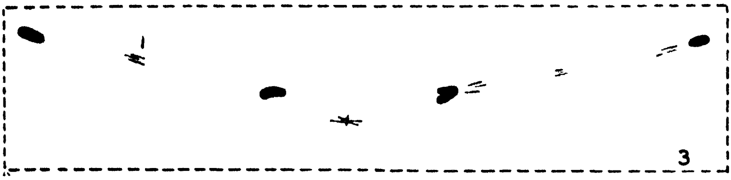
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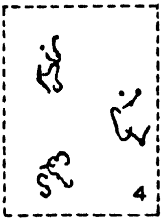
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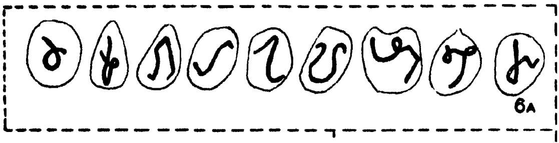
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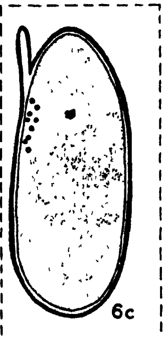
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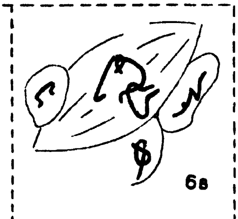
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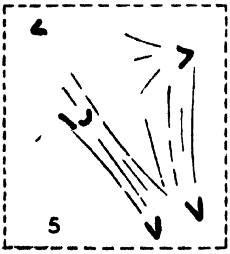
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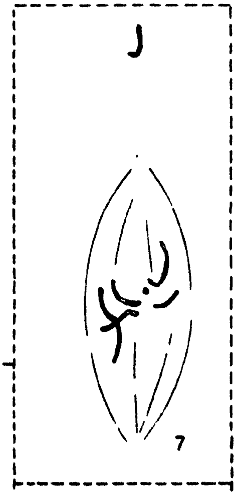
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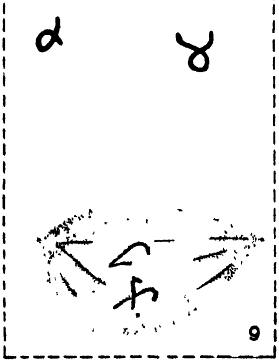
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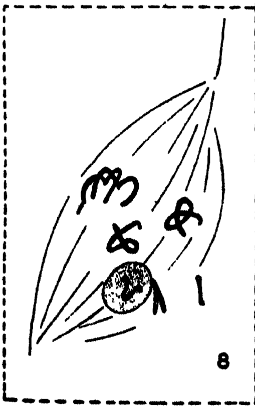
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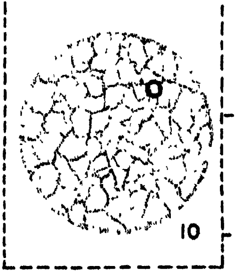
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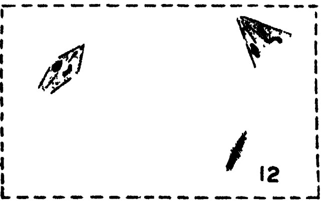
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GENETIC STUDIES ON THE NATURE OF HERMAPHRODITIC PLANTS IN ANTENNARIA DIOICA (L) GAERTN.

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IN MY previous papers on *Antennaria dioica* (1930, 1932, 1934) I dealt with the relations between color and sex, and considered the sex ratio as well as the inheritance of these characters. I made only passing reference to the fact that in addition to typical males and females, intermediate forms exist which may have more or less fertile organs of both sexes. The purpose of this paper is to give a genetic analysis of these aberrant forms.

The first classic paper on Antennaria by JUEL (1900) in which the author succeeded in demonstrating the parthogenetic reproduction of *Antennaria alpina*, contains the description of aberrant forms of *Antennaria alpina* and *A. dioica*, to which he refers in describing *A. dioica* as (1) intermediate hermaphrodites, (2) predominantly female hermaphrodites, and (3) aberrant females. Of these groups, the first resembles very much the typical staminate floret, yet is smaller in all dimensions and has a fertile ovary; the second group has two (instead of five) stamens, which, however, are not grown together to form a tube; the style is not as short and stiff as in the typical male but, on the other hand, does not show the elongated and parted appearance of the typical female; the third group is pure female, resembling only somewhat the staminate florets in the formation of the style and the external parts of the florets. For *A. alpina*, JUEL also describes an aberrant staminate type, which, however, has no pollen grains and no fertile germ cells. The same aberrant form seems to be more frequent than the normal one, for BERGMAN (1935) also recently reported it for *A. alpina*, and STEBBINS (1935) also describes it for the parthenogenetic species *A. fallax*, *A. parlinii* and *A. canadensis* in North America. It seems that in Antennaria aberrant forms are found wherever one makes a careful analysis.

DESCRIPTION OF THE NORMAL AND ABERRANT FLORETS OF ANTENNARIA DIOICA

Before reporting my own experiments with the various sexual forms of *A. dioica*, I shall give a more detailed description of them. The typical female floret of *A. dioica* has a slender pistil ending in two branches which bear stigmatic papillae. The corolla-tube encloses the style very tightly and there are no pollen sacs. The pappus-bristles are thin and their cells are almost confined to one row. The ovary is fertile. Text figure 1A shows the upper part of the corolla and the parted and protruding style. Figure 2 shows the apex of a pappus-bristle. Figure 3A represents a cross-section of the corolla and style.

The typical male floret has a thick button-shaped style which is very little divided or not divided at all. It shows no stigmatic papillae, yet on the external side it bears sweeping-hairs which sweep out the pollen grains when the latter are expelled from the sensitive pollen sacs on the external side of the style. Around the style stand five stamens grown together and forming a tube. This tube in its turn is surrounded by the corolla, which loosely incloses the pollen sacs. The pappus-bristles are broad and multicellular at the apex. Text figure 1H shows the upper part of the male floret. The appendices of the anthers protrude from the corolla. Figure 2G shows the upper part of the pappus-bristles. Figure 3C represents a section across the anthers and the corolla. (All corresponding figures of the different forms are drawn at the same magnification.)

The other pictures in figures 1 to 3 show all transitions between typical female and typical male florets. Figures 1B and 2B correspond to JUEL's aberrant female, a form that occurs very frequently, and that is represented here by plant number 250 (see also figure 3, plate 1). Its stigmata are somewhat stiffer and thicker and the corolla is a little wider; yet the floret is always purely pistillate, fertile, and never shows any evidence of anthers. During the blooming period it is easily distinguished from the typical female. Later, when the stigmata are dry, it may sometimes be mistaken for normal forms. (See plate 1, figures 6 and 7. Figure 6A represents the floret of a typical male, figure 6B of a typical female, and figure 7 of a floret of the same type as plant number 250.)

The predominantly female form as described by JUEL is seen in figures 1C and 1D and in 2C and 2D. It shows its less developed florets, resembling closely the type described above, yet it may contain quite well developed hermaphroditic florets. Figure 1D shows the button-shaped style with sweeping-hairs, while figure 1C in addition to the latter shows stigmatic papillae on the inside. The pappus-hairs (figures 2C and 2D) are somewhat broader than in pure females but much thinner than in pure staminate forms.

The next forms (plant no. 190, figure 1E and plant no. 253, figures 1F and 1G) approach the male type. The florets usually have male appearance although the style is often not enclosed in the anther tube but grows out from one side. The style frequently is bent as seen in figure 1G; self- or cross-fertilization thereby becomes difficult. The pappus-hairs are somewhat thicker than in the previous case. Nevertheless, these types are very similar to each other and can be distinguished only by their inheritance. The latter form probably ought to be put into one class with JUEL's "intermediate hermaphrodites." Figure 3B shows the parts of the floret at the level of the anthers. There are only three anthers which are not joined in a tube; the style is situated on one side, and the corolla is as wide as in the males (plate 1, figure 8 shows two florets of plant 190 and plate 1, figure 5 shows one inflorescence of the same plant).

In addition to these differences, the male and female plants on the whole and the male and female heads in particular show distinct differences. This can be seen, without description, in figure 1, plate 1, where a male is shown at the left, a female at the right, and an aberrant female inflorescence in the center. The involucral bracts are much broader in the male, more elongated in the female. The fact that the color is different in each form and that there is a tendency in the males towards bright and in the females towards dark red colors has been referred to in one of my earlier papers (1930).

PROPORTIONS OF NUMBERS AND INHERITANCE OF THE DIFFERENT TYPES

In my previous papers I have attempted to analyze the numbers in which males and females appear. There are three possibilities: either as many males as females, or a preponderance of either males or females. I found that these proportions are due to a fertility factor which is transmitted together with the gene for sex and which allows a better growth inside the style to those pollen tubes which have at least one factor in common with the style tissue. The following possibilities have to be considered (table 1). The results of 42 crossing experiments are reported in tables 4–6 of my earlier paper (1934). I stated there that in certain crosses about half of the females belong to a more staminate type, but made no special report on them. In tables 2–5 of the present paper all these crosses are reprinted with special consideration of the aberrant forms. In addition, there are recorded 27 new crosses carried out during the last two years. Plants of the former crosses have bloomed for the first time since the last publication; hence, several figures relating to these had to be altered.

If we consider column 5, which contains the number of occurring forms, we find that in a series of cases there appear intersexes in addition to fe-

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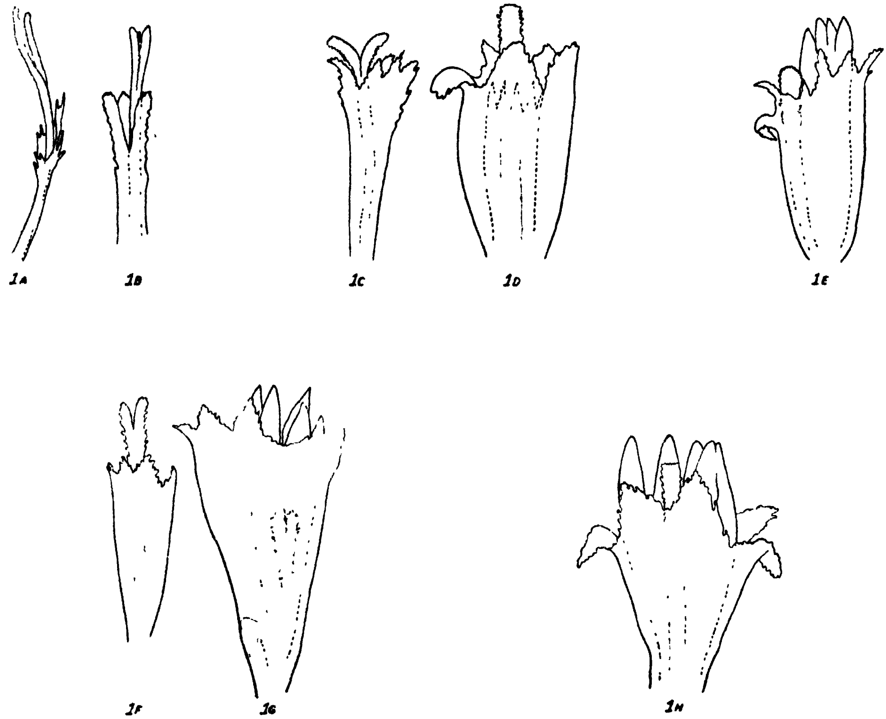
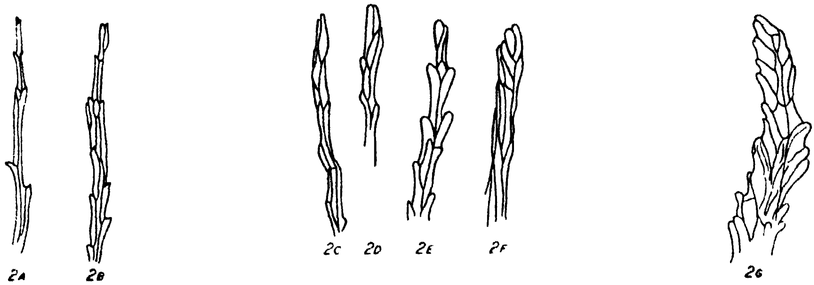
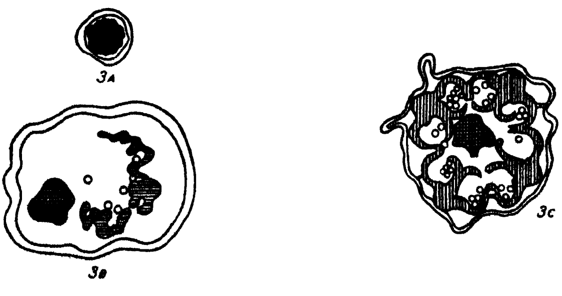
FIGURES 1–3

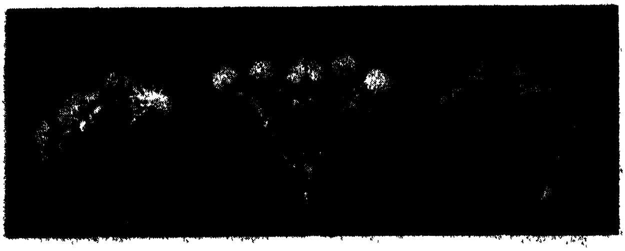
1A.—Floret of typical female, *FSFS*. 16×; 1B.—Floret of aberrant female, *F'SFS*, no. 250. 16×; 1C and 1D.—Florets of predominantly female hermaphrodite, *F'SF'S*, no. 306. 16×; 1E.—Floret of aberrant male hermaphrodite, *FSf'S*, no. 190. 16×; 1F and 1G.—Florets of aberrant male hermaphrodite *FS'f'S*, no. 253. 16×; 1H.—Floret of typical male, *FSfS;* 2A.—Hairbristle of typical female, *FSFS*. 50×; 2B.—Hairbristle of aberrant female *F'SFS*, no. 250. 50×; 2C and 2D.—Hairbristles of predominantly female hermaphrodite *F'SF'S*, no. 306. 50×; 2E.—Hairbristle of aberrant male hermaphrodite *FSf'S*, no. 190. 50×; 2F.—Hairbristle of aberrant male hermaphrodite *FS'fS*, no. 253. 50×; 2G.—Hairbristle of typical male, *FSfS;* 3A.—Cross-section of a female floret. 25×; 3B.—Cross-section of a hermaphrodite floret, no. 190. 25×; 3C.—Cross-section of a male floret. 25×.

Von Ubisch, Genetics of Hermaphroditism in Antennaria Figs. 1–3









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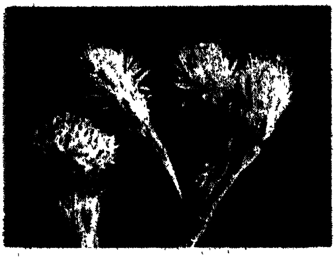

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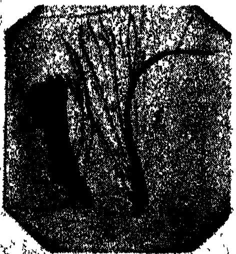

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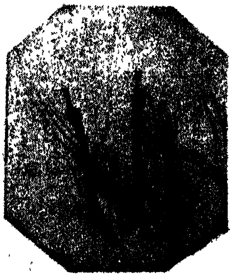

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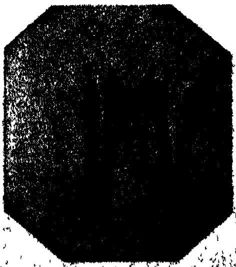

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DESCRIPTION OF PLATE 1

FIGURE 1.—Male, hermaphrodite and female inflorescences of *Antennaria dioica*. Natural size.

FIGURE 2.—Inflorescence of a typical female. Genetic formula *FSFS*. Enlarged.

FIGURE 3.—Inflorescence of an aberrant female, no. 250. Genetic formula *F'SFS*.

FIGURE 4.—Inflorescence of a predominantly female hermaphrodite, no. 306. The head at the left resembles a male. Genetic formula *F'SF'S*.

FIGURE 5.—Inflorescence of an aberrant male hermaphrodite, no. 190. Genetic formula *FSf'S*.

FIGURE 6.—Micro-photograph of typical male floret (left) and typical female floret (right). 5×

FIGURE 7.—Micro-photograph of aberrant female floret, no. 255, *F'SFS*. 5×

FIGURE 8.—Micro-photograph of aberrant male hermaphrodite floret, no. 190, *FSf'S*. 5×

TABLE I

PARENTS		PROGENY							CASE	
FEMALE	MALE	FEMALES			MALES					
		MANY		FEW	MANY		FEW			
FS FS	FS fS	FS FS			FS fS				1.	
FS'FS'	FS fS			FS'FS			FS'fS		2.	
FS'FS'	FS'fS'	FS'FS'			FS'fS'				3.	Same number of both sexes.
FS FS	FS'fS'			FS FS'			FS fS'		4.	
FS'FS	FS fS	FS FS	FS'FS		FS fS	FS'fS			5.	
FS'FS	FS'fS'	FS'FS'	FS FS'		FS'fS'	FS fS'			6.	
FS'FS	FS'fS	FS'FS'	FS FS'		FS fS	FS'fS			7.	
FS'FS	FS fS'	FS FS	FS FS'		FS'fS'	FS fS'			8.	
FS FS	FS fS'	FS FS					FS fS'		9.	Preponderance of females.
FS'FS'	FS'fS	FS'FS'					FS'fS		10.	
FS FS	FS'fS			FS FS'	FS fS				11.	Preponderance of males.
FS'FS'	FS fS'			FS FS'	FS'fS'				12.	

TABLE 2*

Equal number of males and females

1 NO.	2 PARENTS	3 ORIGIN	4 TOTAL NO.	5 ♀ : ⚥ : ♂	6 %♀	7 FORMULAE ♀ ♂	8 TYPE OF FEMALE	9 TYPE OF INTERSEX	10 CASE
287	114×247	Sandh.×Krem.	138	69: 0: 69	50	*FSFS×FSfS*			I, 1
293	153×248	Kremn.×Krem.	212	106: 0:106	50				
515/16	453×442	265×319	209	98: 0:111	47				
512/22	446×442	319×319	85	42: 0: 43	49	*F'SFS×FSfS*	*F'SFS*		I, 2
319	240×308	Sandh.×Mosk.	120	36:26: 58	52	*F'SFS×F'SfS*	*F'SFS*	*F'SF'S*	I, 3
315	255×190	Kremn.×Krem.	89	19:23: 47	47	*F'SFS×FSf'S*		*F'SFS*	I, 4
423	351×307	273×Herlö	37	11: 8: 18	51	*F'SFS'×F'SfS*	*F'SFS'*	*F'SF'S*	V, 1
540	460×477	316×296	103	24:33: 46	55	*F'SFS'×FS'fS'*		*F'SFS'*	VI
222	191×164	Kremn.×Kremn.	18	11: 0: 7	61	*FSFS'×FS'fS*			VII, 1
302	241×164	Sandh.×Krem.	285	76:72:137	52	*F'SFS'×FS'fS*		*F'SFS'*	VII, 2
415	355×307	Herlö×Herlö	84	24:23: 37	56			*F'SFS'*	
304	254×162	Heidesh.×Kr.	74	38: 0: 36	51	*FS'FS×FSfS'*			VIII, 1
203	192×162	Kremn.×Kremn.	108	51: 0: 57	47	*F'S'FS×FSfS'*	*F'S'FS*		VIII, 2
204/20	192×172	Kremn.×Krem.	269	146: 0:123	54		*F'S'FS*		
281	59×242	Brünn×Sandh.	213	97:12:104	52		*F'S'FS*		

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* The number appearing in the first column of the tables, for instance 287, refers to the number of the experiment. Experiments 200–222 were sown in 1928, 260–275 in 1929, 280–340 in 1930, 400–427 in 1932, and 500–558 in 1933. During the first year after the sowing only a few plants flower, the entire blooming period extending over many years. There is no reason to assume that wrong proportions of sexes were obtained during the first years (if we ignore the error due to smaller numbers), for as shown in a small table (1930) the first blooming of males and females occurs in the same proportions. In column 2 are recorded the numbers of the parents, as for instance 114×247, the female first. Column 3 gives the origin of the parents, either the place where they were collected (Sandhausen near Heidelberg; Heidesheim near Mayence; Kremnica and Brno in Czechoslovakia; Orellen in the Baltic; Moscow in Russia; Fellhorn in the Bavarian Allgaeu, and Herlö in Norway), or, in the case of plants resulting from a former cross, the number of this cross; thus, 269×319 indicates that the female which was used came from cross 269, the male from cross 319. Column 4 gives the total number of plants; column 5 the number of females, hermaphrodites and males; column 6 the number of females, the hermaphrodites being included among them; column 7 the genetic formula, and column 10 gives the key for each case with reference to table 1 of this paper. In columns 8 and 9 the type is recorded to which the females belong (if they are not *FF*) and the intersexes.

TABLE 3

Preponderance of females

1 NO.	2 PARENTS	3 ORIGIN	4 TOTAL NO.	5 ♀ : ⚥ : ♂	6 % ♀	7 FORMULAE ♀ × ♂	8 TYPE OF FEMALE	9 TYPE OF INTERSEX	10 CASE
208	113×160	Sandh.×Krem.	62	44: 0: 18	71	*FSFS*×*FSfS'*			IX, 1
207	113×162	Sandh.×Krem.	75	52: 0: 23	69				
280	52×243	Brünn.×Sandh.	15	15: 0: 0	100				
282	66×177	Brünn×Sandh.	185	185: 0: 0	100				
300	239×177	Kremn.×Sand.	310	280: 0: 30	90				
295	165×175	Kremn.×Sand.	226	194: 0: 32	86				
206/91	130×160	Sandh.×Kr.	294	204: 0: 90	69				
289	114×162	Sandh.×Kr.	62	36: 0: 26	58				
211	131×162	Sandh.×Kr.	31	31: 0: 0	100				
538	458×476	Orellen×Hö	108	96: 0: 12	89				
272	255×175	Kremn.×Sdh.	85	61: 0: 24	72	*F'SFS*×*FSfS'*	*F'SFS*		IX, 2
268	251×242	Kremn.×Sdh.	101	63: 0: 38	62		*F'SFS*		
269	251×162	Kremn.×Krem.	58	56: 0: 2	97		*F'SFS*		
283	110×162	Sandh.×Kr.	200	198: 0: 2	99		*F'SFS*		
265	250×242	Kremn.×Sdh.	404	310: 0: 94	77		*F'SFS*		
213	110×160	Sandh.×Sandh.	14	13: 0: 1	98		*F'SFS*		
500/1	447×440	319×220	264	121: 86: 57	79			*F'SFS*	
502/3	448×440	319×220	467	209:179: 79	83			*F'SFS*	
507	455×441	265×265	30	9: 10: 11	63			*F'SFS*	
523	481×441	283×265	17	8: 6: 3	83			*F'SFS*	
528/30	483×440	283×220	38	9: 24: 5	87			*F'SFS*	
413	306×257	Herlö×203	86	82: 0: 4	95	*F'SF'S*×*FSfS'*	*F'SFS*		IX, 3
284	110×196	Sandh.×Fell.	311	94:168: 49	84	*F'SFS*×*F'SFfS'*	*F'SFS*	*F'SF'S*	IX, 4
294	161×196	Kremn.×Fell.	84	34: 50: 0	100		*F'SFS*	*F'SF'S*	
301	240×196	Sandh.×Fell.	386	170:104:112	71		*F'SFS*	*F'SF'S*	
401	349×156	253×Kremn.	29	12: 17: 0	100	*FS'F'S'*×*FS'fS*		*FS'F'S'*	X, 1
403	341×307	253×Herlö	6	4: 2: 0	100			*FS'F'S'*	
406	350×345	253×275	91	38: 33: 20	78			*FS'F'S'*	
407	350×307	253×Herlö	468	249:125: 94	80			*FS'F'S'*	
408	350×156	253×Kremn.	593	178:174:241	60			*FS'F'S'*	
409	343×307	253×Herlö	22	9: 13: 0	100			*FS'F'S'*	

TABLE 4

Preponderance of males

1	2	3	4	5	6	7	8	9	10
NO.	PARENTS	ORIGIN	TOTAL NO.	♀ : ⚥ : ♂	%♀	FORMULAE ♀ ♂	TYPE OF FEMALE	TYPE OF INTERSEX	CASE
285	113×156	Sandh.×Krem.	100	44: 0: 56	44	*FSFS×FS'fS*			IX, 1
209/10	113×164	Sandh.×Krem.	165	63: 0:102	38				
202	130×156	Sandh.×Krem.	152	58: 0: 94	38				
201	130×164	Sandh.×Krem.	81	16: 0: 65	20				
286/88	114×106	Sandh.×Sand.	40	16: 0: 24	40				
217/90	114×164	Sandh.×Sand.	67	17: 0: 50	25				
508/9	445×156	300×Kremn.	19	6: 0: 13	31				
273	255×156	Kremn.×Krem.	39	14 25	36	*F'SFS×FS'fS*		*F'SFS'*	XI, 2
316	255×253	Kremn.×Krem.	108	26:21: 61	43	*F'SFS×FS'f'S*		*F'SFS'*	XI, 3
266	110×253	Sand.×Kremn.	48	20: 0: 28	42		*F'SFS'*		
314	306×253	Herlö×Kerm.	16	8 8	50	*F'SF'S×FS'f'S*			XI, 4
536	485×461	207×316	50	5: 0: 45	10	*FSFS×FS'f'S*			XI, 5
296	167×196	Kremn.×Fellh.	233	85 148	36	*FS'FS'×F'SfS'*			XII

males, usually in nearly the same number. If we add these to the females, a percentage of females is obtained (column 6) which corresponds to the figures of column VI in my earlier paper (1934). These intersexes are the aberrant forms described in this paper.

TABLE 5

Self fertilization

1	2	3	4	5	6	7	8	9	10
NO.	PARENTS	ORIGIN	TOTAL NO.	♀.⚥:♂	%♀	FORMULAE ♀ ♂	TYPE OF FEMALE	TYPE OF INTERSEX	CASE
551	469	315	40	12: 7:21	30	$FSf'S \times FSf'S$		$FSf'S$	
544/45	463	315	44	11:11:22	25			$FSf'S$	
549	467	315	10	3: 2 5	30			$FSf'S$	
546	464	315	20	0:11: 9	0			$FSf'S$	
547	465	315	11	6: 2: 3	55			$FSf'S$	
550	468	315	9	3: 3: 3	33			$FSf'S$	
548	466	315	12	8: 1:13	67			$FSf'S$	
543	462	316	53	23:11:19	44			$FSf'S$	
542	461	316	12	4: 4: 4	33			$FSf'S$	
417	253	Kremn.	19	7: 7: 5	37			$FS'f'S$	

TABLE 6

Melandrium dioicum	*Antennaria dioica*
$F'F':MM$ = 120:80 superfemales	$F\,F\,:MM$ = 100:80 typical females
$F\,F':MM$ = 110:80 superfemales	$F\,F':MM$ = 95:80 female intersexes
$F\,F\,:MM$ = 100:80 typical females	$F'F':MM$ = 90:80 female intersexes
$F'f'\,:MM$ = 84:80 male intersexes	$F\,f'\,:MM$ = 73:80 male intersexes
$F'f\,:MM$ = 80:80 male intersexes	$F\,f\,:MM$ = 70:80 typical males
$F\,f'\,:MM$ = 74:80 male intersexes	$F'f'\,:MM$ = 68:80 inframales
$F\,f\,:MM$ = 70:80 typical males	$F'f\,:MM$ = 65:80 inframales
$f'\,f'\,:MM$ = 48:80 inframales	$f'\,f'\,:MM$ = 46:80 inframales
$f'\,f\,:MM$ = 44:80 inframales	$f'\,f\,:MM$ = 43:80 inframales
$f\,f\,:MM$ = 40:80 inframales	$f\,f\,:MM$ = 40:80 inframales

If we attempt now to give a genetic analysis of these forms, we may follow the explanation of G. and P. HERTWIG (1922) regarding the intersexes of Melandrium. This dioecious plant occasionally produces also hermaphroditic forms, but they are transformed males, as is shown by their anatomical structure. The HERTWIGS gave the following explanation of the forms. Since Melandrium is heterozygotic in the male, we must ascribe the formula $FFMM$ to the female and $FfMM$ to the male. The quantity of the female factor must hence be greater than that of the male factor, for instance, F:50; M:40; f:20. Thus in typical females, the female:male ratio $FFMM$ = 100:80; in typical males, the female:male ratio $FfMM$ = 70:80. There might be an increase of the quantities from

F to F' and from f to f' of 20 percent. In this case we have the following possibilities (table 6, left). Some of these aberrant forms could be identified by the HERTWIGS. The difficulty with their material as well as with mine lies in the wide range of variation. Inframales could not be obtained; they are probably inviable.

From the formulae for the intersexes it may be seen that they originate from the males since they all have f. This distinguishes them from the Antennaria intersexes.

HYPOTHESIS TO EXPLAIN THE ANTENNARIA INTERSEXES

The typical female is given the formula of $FFMM$ with $F=50$, $M=40$. The typical male is $FfMM$, f being 20 as in Melandrium. We assume a quantitative alteration from F to F', diminishing the quantity of F to $F'=45$, while the quantity of f is raised to $f'=23$. (There is no reason to raise or to diminish the quantities of F and f proportionately, as their alterations must have been brought about by independent mutation.) Thus we obtain the sexual forms of table 6, right. The aberrant female will then be $F'F'MM$ and the intermediate hermaphrodite $Ff'MM$. Only the latter is derived from the male form. In contrast to Melandrium here occur also $f'f'MM$ forms. All males resulting from self-fertilization must have this formula (table 5), while the HERTWIGS did not get plants, definitely known to be males, from self-pollination of hermaphrodites.

Considering tables 2–5 from the point of view of the genetic formulae, the fact must be stressed that the deviation from the chance sex ratio 1:1 is in no way altered by the modification of the quantity of the factors F and f into F' and f', if the aberrant forms are classified with the female and male forms respectively, according to whether they are $F'F$ and $F'F'$ or Ff'. The sex ratio depends on the fertility factor S or S' only (table 1). The proportions of typical to aberrant pistillate forms ought to be 1:1 whenever it appears. Any deviation must be ascribed to the range of variation which obscures the differences. It is always easier to state that there are two different types in the crosses than it is to classify the plants into these types. Thus in the crosses the FF' are considered typical female if together with them appear $F'F'$ females which were recorded as aberrant females. In other crosses $F'F$ was considered as aberrant form when no $F'F'$ appeared. Besides, the eye had to become accustomed to the subtle differences; in the first years (numbers below 304) $F'F$-flowers on account of these difficulties were considered as typical in most instances. Finally, the plants of the last year (1935) were sent to me dried, and in this condition small differences cannot be distinguished.

If in view of this, the assigned genetic formulae seem to be questionable, I should like to draw attention to some invariable features of the classification: 1. $F'F'MM$-forms were always considered as intersexes. 2. When

F'FMM and *F'F'MM* appeared together, the former were always considered as typical, the latter as intersexes. Crosses with the recently obtained male form *F'fMM* should be especially interesting. This form is considered an intersex by the HERTWIGS; in Antennaria it is an inframale. Crossed with normal females, however, intersexes must result, and a cross with the intermediate form *FF'MM* must give rise to hermaphrodites. The actual occurrence of these postulated types is demonstrated by one individual each in cases I,3, V,1, and XII, and by three individuals in case IX,4 (tables 2–4).

The correctness of our assumption seems assured by the great number of crosses with relatively few different plants, for which all crosses must show the same genetic formula. There are 69 crosses with 36 different plants: each plant appears in an average of four crosses, sometimes much oftener, and the genetic formula is seldom in doubt.

No information is available concerning the origin of the aberrant factors *F'* and *f'*. My first assumption that the factors would be somewhat different in the various countries, and that we are dealing with different geographical races in the sense of RICHARD GOLDSCHMIDT, has proved correct only in so far as the plants from Norway, Moscow and Allgaeu perhaps had a somewhat different quantity than the others, but the numbers are not sufficient to justify the assumption of factors *F''*, *f''*, etc. The plants from Central Europe were alike in that they showed both normal and aberrant forms.

A necessary complement of the analysis is the examination of the chromosomes, which I expect to finish during the next years as I possess fixed material from almost every plant. The chromosome number of *Antennaria dioica* has been determined by JUEL (1900) to be 12–14 haploid, the same result being obtained by BERGMAN (1935) who counted 28 chromosomes in the root-tips. My own rather superficial examinations show that in the embryo-sac and in the pollen mother cells chromosomes of very different forms and types appear which will perhaps make it possible to distinguish one of the chromosomes as the sex chromosome. In this case it remains to be determined whether and how the intersexes differ cytologically from the normal forms.

SUMMARY

In *Antennaria dioica* (L) Gaertn. a number of forms occur which according to their structure stand between the normal female and the normal male or surpass the latter. An explanation on a genetic basis is given for these forms and their inheritance investigated in a number of crosses.

I am very much indebted to Miss Lilli zur Nieden who enabled me to finish this investigation by carefully reaping and sending to me the plants which had flowered during 1935, and to Mrs. Anny Planet for her help with the English text.

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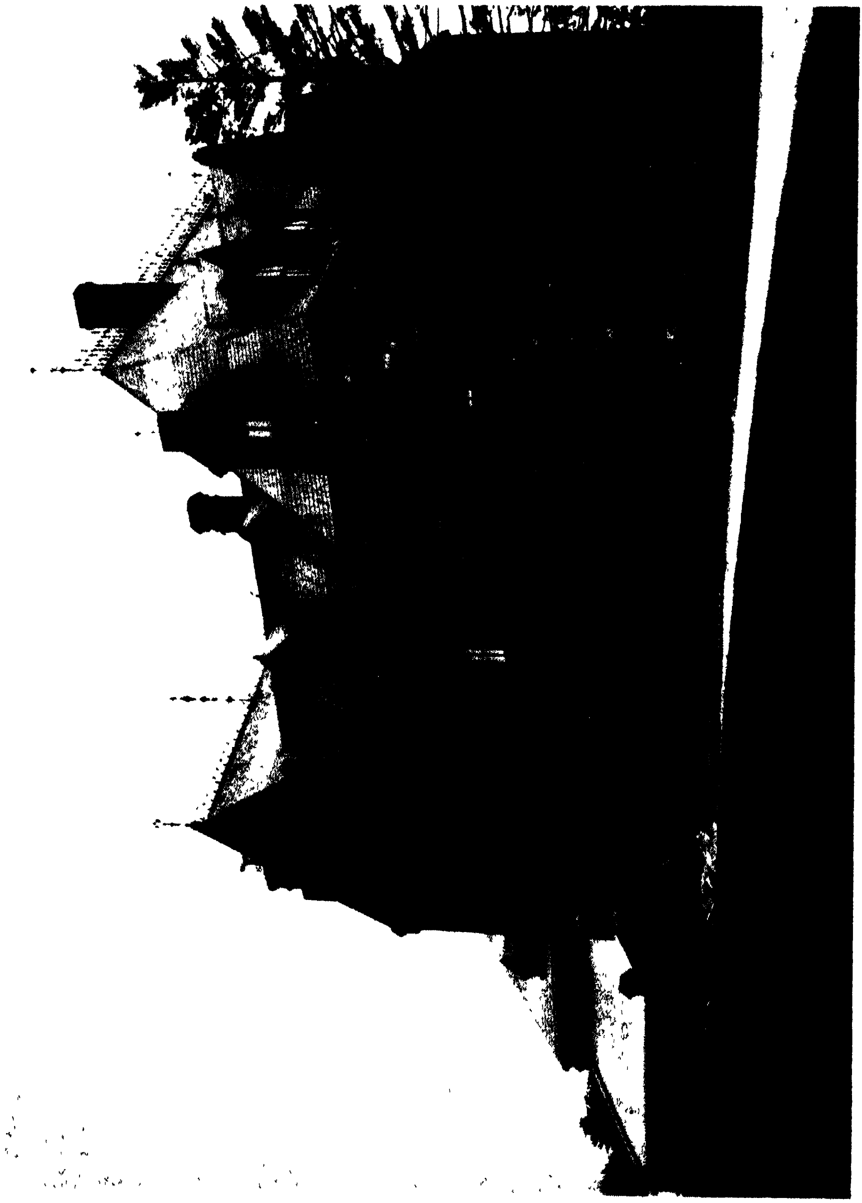

THE BUSSEY INSTITUTION OF HARVARD UNIVERSITY

Founded 1872—Closed June 30, 1936

THE Bussey Institution was founded in 1872, as a school of agriculture, and was named after BENJAMIN BUSSEY, on whose farm in Jamaica Plain the Institution was located. BENJAMIN BUSSEY'S will, drawn in 1835, provided for a school of agriculture and horticulture and anticipated, by more than twenty-five years, the Morrell Act of Congress, establishing the State Agricultural Colleges. The actual organization of the Bussey Institution was begun during the first year of President Eliot's administration (1869), and the building was completed in 1872.

The original faculty consisted of DEAN STORER, who taught agricultural chemistry; FRANCIS PARKMAN, as professor of horticulture; THOMAS MOTLEY, as instructor in farming; D. D. SLADE, as professor of applied zoology; and G. F. SANBORN, as instructor in entomology. The first contribution in plant breeding from the Bussey Institution was an article on "Hybridization of Lilies," published in the Bulletin of the Bussey Institution by FRANCIS PARKMAN, the famous historian.

PROFESSOR FARLOW began his work at the Bussey Institution in 1875, followed by PROFESSOR GOODALE and C. E. FAXON. FARLOW and GOODALE moved to Cambridge in 1879. In 1895 DR. THEOBALD SMITH was appointed to the professorship of applied zoology. He became affiliated with the Medical School and established the Antitoxin Laboratory, which was erected in 1903.

The Bussey Institution income was always meagre, but the early history is especially depressing. During 1879–80 DEAN STORER received $500, and several members of the staff received no salary. For many years the farm was used for boarding horses and cattle, and to provide vegetables for the students' dining hall at Harvard. The students paid for their tuition by cutting wood, which was sold to the professors in Cambridge.

The Bussey Institution functioned as an undergraduate school of agriculture for about thirty years, and was then reorganized as a graduate school in applied biology. PROFESSOR W. M. WHEELER was called from the Natural History Museum in New York to take charge of this work in 1908. The same year PROFESSOR W. E. CASTLE established his work in animal genetics at the Bussey Institution, and a year later PROFESSOR E. M. EAST was appointed to take charge of plant genetics. PROFESSOR C. T. BRUES joined the entomology department in 1910. In 1914 the department of forestry was affiliated with the Institution, and work in plant anatomy was started by PROFESSOR I. W. BAILEY. A year later economic botany, under the direction of PROFESSOR OAKES AMES, was included. PROFESSOR AMES maintained his laboratory in the Bussey until 1926, when he moved to Cambridge as Curator of the Botanical Museum. In 1930 the Bussey faculty was merged with the faculty of arts and sciences, and the instructional and research work in entomology was transferred to Cambridge.

Research work in genetics and plant anatomy was continued at the Bussey for the next six years. The limited income made it necessary to close the Bussey in June, 1936. PROFESSOR CASTLE retired, after having served on the Harvard faculty since 1897, and the other members of the Bussey faculty were transferred to the new Biological Institute in Cambridge. The Bussey greenhouses and breeding plots have been retained for experimental work in plant genetics and cytology.

Since the reorganization of the Bussey as a graduate school, forty geneticists have received the degree of Doctor of Science, twenty in animal genetics and twenty in plant genetics. Nearly all these graduates are actively engaged in genetic research. Most of them hold important positions in various universities, federal bureaus, or agricultural experiment stations in the United States. A still greater number of geneticists have worked at the Bussey for shorter periods of time. Many of these men were post-doctorate students on traveling fellowships from foreign countries.

The Bussey Institution has given rise to three vigorous offspring: the Arnold Arboretum, the Antitoxin Laboratory of the State Board of Health, and, indirectly, the new Biological Institute. The work in genetics will be continued at the Biological Institute.

DOCTORATES CONFERRED BY HARVARD UNIVERSITY FOR WORK IN GENETICS AT THE BUSSEY INSTITUTION

Mammalian Genetics

J. A. Detlefsen
E. C. MacDowell
C. C. Little
Sewall Wright
L. C. Dunn
W. L. Wachter
Tage Ellinger
H. W. Feldman
W. H. Gates
L. H. Snyder
C. E. Keeler
G. W. Hervey
Gregory Pincus
P. W. Gregory
E. A. Livesay
R. C. Robb
Paul B. Sawin
N. F. Waters
F. H. Clark
S. C. Reed

Plant Genetics

R. A. Emerson
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J. B. Park
G. F. Freeman
D. F. Jones
E. F. Gaines
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Edgar Anderson
Karl Sax
R. A. Brink
H. C. McPhee
P. C. Mangelsdorf
W. S. Hsu
A. J. Mangelsdorf
W. R. Singleton
S. H. Yarnell
F. A. McCray
H. H. Smith
E. R. Sears

STUDIES OF INHERITANCE IN LOP-EARED RABBITS

W. E. CASTLE AND S. C. REED

Bussey Institution, Harvard University

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IN 1909 CASTLE published some observations on the behavior of ear length in crosses between lop-eared and ordinary varieties of rabbits, describing the inheritance as blending. Shortly thereafter LANG (1911) applied to these observations the multiple factor hypothesis which had been formulated by NILSSON-EHLE on the basis of his studies of quantitative characters in oats and wheat. Since then, numerous studies of quantitative characters in animals and plants have served to establish firmly the multiple factor hypothesis. But no further study has been made of the peculiar ear character found in lop-eared rabbits. CASTLE has meanwhile shown that among other varieties of rabbits there is a close correlation between general body size and ear length, skull length, and leg length. During development, the body as a whole and its various parts grow faster lengthwise than transversely. So the general body form, and that of all elongated organs, becomes more slender as the absolute size increases. Accordingly, other things being equal, the larger the rabbit, the longer proportionally are its ears, its skull, and its legs.

Lop-eared rabbits are one of the oldest recognized varieties of domestic rabbits and have undoubtedly attained their present excessive ear length as a consequence of long-continued selection. Other breeds of rabbits, for example Flemish Giants, exceed them in size of body, but none in length, width, and softness of the ears. This last characteristic (softness) they owe to a deficiency of cartilage in the ear, which makes it pliable and loppy and incapable of erection, as are the ears of ordinary rabbits. The body size of standard bred lop-eared rabbits is large, and that is one reason why their ears are long; but their ears are much longer than the ears in other breeds of rabbits of substantially the same body size. This shows that some additional agency operates to make the ears of lop-eared rabbits long. That it is a genetic agency there can be no doubt, for lop-eared rabbits are known to be true breeding, and their peculiar character in crosses is transmitted equally through male and female individuals in the blending manner generally recognized as indicating the operation of multiple genes.

In the light of what has been learned about heredity in the last highly fruitful quarter century, it has been thought desirable to make new observations on the results of crossing lop-eared rabbits with ordinary breeds of rabbits. To this end, in March 1932, a trio of purebred lop-eared rabbits was obtained from Mr. T. Corbishly, Secretary of the Lop-Eared

Rabbit Club of England, through arrangements kindly made by Mr. E. C. Richardson.

The results to be described in this paper were obtained by (a) crossing the lop-eared male with females of other breeds; (b) raising an F_2 generation from F_1 parents; (c) backcrossing F_1 females to the lop-eared male; or (d) backcrossing F_1 individuals to the other parental (short-eared) stock.

METHODS AND STOCKS EMPLOYED

It has been our endeavor to grow the experimental rabbits under optimum conditions. To this end a mother has not been allowed to suckle more than 4 or 5 young at a time, the remainder of the litter, if any, being entrusted to foster mothers. The young have been weaned, as a rule, when one month of age. At that time each animal was weighed, and measurements were made of its ear length and ear width. Thereafter the animals were weighed and had their ears measured once every two weeks until they were four months old. At this age growth of the ears is nearing completion, although growth of the body may continue to the age of a year, or even longer.

As a basis for comparing individuals of different generations or litters, an estimate was made of the weight and ear measurement of each individual at the age of 120 days. Ear lengths were measured with a thin ruler from the base of the ear to its tip, the ear being held vertical and slightly stretched. Three or four independent readings were made at each measuring to minimize observational errors. At 120 days rabbits of small to medium body size will have practically completed their ear growth. The ears of rabbits of larger body size will continue to grow at a steadily diminishing rate for a few weeks longer, but the proportional change will be small and may safely be disregarded for statistical purposes. The lop-eared male was said to be about ten months old when we received him in March 1932. He then weighed about 4,000 grams, and his ear length was 25.5 cm. At four months old he probably had an ear length of about 24.0 cm, judging by the growth rates of our 3/4 blood lop rabbits. We shall accordingly rate him as a 24.0 cm rabbit.

He was mated with females of several different breeds, such as Blue Beveren, New Zealand Red, and the synthetic English-Dutch race of CASTLE. These range in body weight between 3,000 and 4,000 grams and in ear length from 11.0 to 13.5 cm. He was also mated with rabbits of smaller size, Himalayan and Polish derivatives. These ranged in weight from 2,000 to 3,000 grams and in ear length from 10.0 to 10.9 cm.

THE F_1 GENERATION

Eleven females in matings with the lop-eared male produced a total of 65 F_1 young, which were reared to maturity. The ear length of these in

MATING	EAR LENGTH OF MOTHER	11.5	12	12.5	13	13.5	14	14.5	15	15.5	16	16.5	17	17.5	18	18.5	19	19.5	NO.	MEAN	S.D.	C.V.
A	10.0	1					1	2	3	1									8	14.57	1.17	8.0
B	10.2							1	6										7	15.15		
C	10.4							2	1		1								4	15.25		
D	10.4									2	2		1	1					6	16.48		
E	10.9								1	1			1	2					5	16.66		
	Total (A–E)	1					1	5	11	4	3		2	3					30	15.53	1.22	7.8
F	11.5										1		3	2					6	17.21		
G	11.8								1	1	1		1	1	1				6	16.70		
H	12.2										2	3	4	1					10	16.85		
I	12.0										1		1		1	1			4	17.50		
J	13.4												1	1	2				4	17.87		
K	13.5										1		1		1	1		1	5	17.98		
	Total (F–K)								1	1	6	3	11	5	5	2		1	35	17.23	.95	5.5

TABLE 2

Variation in ear length of the F_2 animals at age 4 months

MATING (SEE TABLE 1)	12.5	13	13.5	14	14.5	15	15.5	16	16.5	17	17.5	18	18.5	19	19.5	20	NO.	MEAN	S.D.	C.V.
♀A×♂A	1		2	1		2		3									9	14.61		
♀E×♂A				1	1			3				1					6	16.10		
♀D×♂D				1		1		1		1							4	15.67		
Total	1		2	3	1	3		7		1		1					19	15.16	1.33	8.8
♀♀C and E×♂H						2	4	5	1	3	3	1					19	16.48		
♀H×♂H			1	1	1	1	5	11	5	11	3	6	1	1			47	16.72		
♀♀I and J×♂H					1		1	3	2		2	5	1	1		1	17	17.46		
♀C×♂I						2	3	1	2	2	3	1	5	1			20	17.24		
♀E×♂I								3	1	2	2	5	1	1			15	17.64		
♀♀F–K×♂I							1	1	3	5	8	2	5	3			28	17.75		
Total			1	1	2	5	14	4	14	23	21	20	13	7		1	146	17.17	1.18	6.8

relation to that of their mothers is shown in table 1. In general, the greater the ear length of the mother, the greater the ear length of the offspring, all having the same father, the lop-eared male with an ear length of 25.5 cm when full grown. One F_1 individual was peculiar in having ears only slightly longer than those of its mother and not at all lopped. This animal (♀ 2994) at 4 months of age had an ear length of 11.6 cm, at which measurement it remained permanently. The mother of ♀ 2994 came of a purebred Himalayan family, and the seven sibs of the peculiar individual were very uniform in ear length (see table 1, Mating A); but this one aberrant individual had singularly short ears and transmitted that peculiarity to her offspring, as we shall see.

In general the ear length of the F_1 animals is nearer to that of the small races. This is not a matter involving superior maternal influence, since in a litter produced by the reciprocal cross (lop-eared ♀ × normal ♂) the F_1 young had similar ear length.

THE F_2 AND BACKCROSS POPULATIONS

An F_2 population was produced consisting of 165 individuals. Of these, 19 resulted from matings within the first group of F_1 individuals (matings A–E). Their variation in ear length is shown in the first section of table 2, the mean being 15.16 cm. The mean for the 146 F_2 individuals classified in the second section of table 2 is 17.17 cm. As compared with the 19 F_2 individuals of the first section of table 2, these are larger animals and have longer ears, as might be expected from their ancestry.

A backcross of F_1 females to the pure lop male (their father) produced a population of 68 animals which are listed in table 4 as 3/4 lop. Twenty-three of these, descended from a small-sized grandmother (matings A and E, table 1), had an average ear length of 19.20 cm. The remaining 45 were descended from larger mothers (matings F–H, table 1). They had an average ear length of 20.07 cm.

Backcrosses of F_1 animals to the maternal parent stock produced the 239 rabbits listed in table 4 as 1/4 lop. The peculiar F_1 female (2994) which had an ear length of only 11.6 cm, produced 18 backcross young. They form a compact group, varying closely about a mean of 10.98 cm, which is intermediate between the ear length of their mother (11.6 cm) and that of their Himalayan father (9.9 cm). The litter mate sisters of this female, likewise from mating A but having ears close to 15 cm long, produced 71 backcross young. They vary about an average of 12.09 cm, which again is intermediate between the ear lengths of the respective parents, 15 and 9.9 cm. The peculiar short ear character of ♀ 2994 would not appear to have been due to a single inhibiting or modifying factor derived from her mother, for in that case her backcross young should show a bimodal distribution owing to segregation on the part of the inhibiting factor. But in reality her backcross young are less variable than the young of her sisters (row 2, table 4), since their standard deviation was only .56 cm and their coefficient of variation 5.1; whereas her sisters' young had a S.D. of .76 cm and a C.V. of 6.2.

All backcrosses to the smaller Himalayan race, when combined, show an average ear length of 11.86 cm; all backcrosses to larger females show an average ear length of 14.42 cm. The coefficient of variation for the two groups is substantially the same (see table 4).

DISCUSSION

The F_1 young of small race mothers are more variable in ear length than the F_1 young of large race mothers, the former having a coefficient of variability of 7.8, whereas that of the latter group is 5.5. This indicates that the small mothers were less homogeneous than the large ones in regard to factors influencing ear length. The F_2 generation bears out this interpretation. The F_2 group from small mothers has a C.V. of 8.8, whereas the group from large mothers has a C.V. of 6.8. The backcross to the pure lop-eared male has a similar implication. The young descended from small mothers have a C.V. of 6.6; the corresponding group derived from large mothers has a C.V. of 5.7. CASTLE (1922) has shown that among ordinary (non-lop) rabbits a close correlation exists between body weight and ear length (.836 ± 0.011). In the present investigation body weights and ear measurements were taken of 67 adult individuals as diverse in size as could be found in the laboratory at that time, ranging in weight from 1,400 to 4,700 grams. In this case also a fairly high correlation was found between body weight and ear length, namely, 0.76 ± 0.03. This indicates that among ordinary rabbits variation in ear length occurs chiefly as a consequence of variation in general body size. The genetic agencies which produce large body size automatically produce long ears and *vice versa*. But the case is very different with lop-eared rabbits. Here a body size of 4,300 grams, which among ordinary rabbits would be associated with an ear length of about 13.5 cm, is found associated with an ear length some 12 cm greater, an increase of 90 percent. We are concerned with investigating what genetic agency has caused this remarkable change.

It might be expected that so great a change in the size of the ear would be attended also with some change in its shape, but this apparently is not true. The relation of ear width to ear length remains about the same, irrespective of the absolute size of the ear. In the 67 adult individuals of various races, the ear length of which we measured, the ratio of ear length to ear width varied closely about a mode of 56 percent, regardless of absolute size (table 3). In a group of twelve 3/4 blood lop rabbits, the group, of all those raised by us, having maximum ear size, the variation was very similar in character and the mean identical at .554.

TABLE 3

Ratio, ear length to ear width

	.51	.52	.53	.54	.55	.56	.57	.58	.59	.60	Mean
Frequency, mixed group	3	4	5	7	12	19	10	3	2	2	55.4
Frequency, 3/4 lop group			1	2	3	3	3				55.4

In the growth of the ear, as well as in its completed state, the relation of length to width changes very little from one month of age to maturity. Previous to one month, change in ear shape is more noticeable.

The twelve 3/4 blood lop individuals whose length-width ratios are recorded in table 3, gave the following average ratios at earlier ages.

Age in months	1	1 1/2	2	3	4	5	6
Mean ratio	59.03	58.03	57.60	57.10	56.32	56.34	55.91

The change in the shape ratio was small and gradual between the ages of 1 and 6 months, amounting in all to only 3 percent. If, instead of calculating such ratios, ear lengths are directly plotted against ear widths on a logarithmic scale for the period between one and six months of age, lines apparently perfectly straight are obtained. Both methods indicate that no genes affecting shape independently of size are in evidence during the period of maximum growth.

It would seem that all races of ordinary rabbits, irrespective of body size, would be equally available for crossing with pure lop-eared rabbits in a study of the genetic agencies responsible for the lop character, since all show a similar relation between body size and ear length. The genetic agency responsible for the added ear length of lop rabbits seems entirely lacking in ordinary rabbits, which differ in ear length chiefly because they differ in body size. If, accordingly, we can devise a means for equalizing the body weights of all rabbits studied, we can estimate what corresponding ear length each would be expected to have.

The regression of ear length on body weight was calculated for the 67 adult non-lop rabbits already mentioned. It was found that ear length increases one millimeter for each increase in weight of one hundred grams. The average body weight for the population of F_1 lop rabbits at four months of age was 2,400 grams. If all animals listed in table 4 had this same body weight, their ear lengths would be directly comparable. Accordingly an ear length was calculated for each individual on this basis, by use of a

TABLE 4

Actual ear lengths at four months of age of the various populations produced by crossing ordinary rabbits with a lop-eared individual

MATING	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15	15.5	16	16.5	17	17.5	18	18.5	19	19.5	20	20.5	21	21.5	22	NO	MEAN	S.D.	C.V.
F_1, A–E				1					1	5	11	4	3		2	3										30	15.53	1.22	7.8
F_1, F–K											1	1	6	3	11	5	5	2		1						35	17.23	.95	5.5
F_2, A–E						1		2	3	1	3		7		1		1									19	15.16	1.33	8.8
F_2, C–K								1	1	2	5	14	22	14	22	19	19	14	7		1					146	17.17	1.18	6.8
1/4 Lop, A ♀ 2994	2	6	6	3	1																					18	10.98	.56	5.1
1/4 Lop, sisters of ♀ 2994		2	11	19	17	13	5	3		1																71	12.09	.76	6.2
1/4 Lop, F–K					2	3	15	29	34	26	17	15	7	2												150	14.42	.92	6.3
3/4 Lop, A–E														3	1	1			6	4	5	3				23	19.20	1.28	6.6
3/4 Lop, F–K														1			3	2	7	6	6	11	5	1	3	45	20.07	1.15	5.7

TABLE 5

Calculated ear lengths at four months of age of the populations of Table 4, if all had the same body weight, 2,400 grams

	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15	15.5	16	16.5	17	17.5	18	18.5	19	19.5	20	20.5	21	21.5	22	TOTAL	MEAN	S.D.	C.V.
P_1, Parents A–E		4	1																						5	11.10		
P_1, Parents F–K			1	1	1	2	1																		6	12.58		
F_1, Matings A–E			1						3	12	5	4	1	2	2										30	15.36	1.08	7.0
F_1, Matings F–K											2	4	7	9	7	5	1								35	16.99	.74	4.4
F_2, A–E					1			4	2		3	4	2	1	1	1									19	15.45	1.36	8.8
F_2, C–K								1	1	4	8	25	17	19	19	23	19	9		1					146	17.16	1.14	6.6
1/4 Lop, ♀ 2994, Mating A	3	9	5	1																					18	11.11	.39	3.5
1/4 Lop, sisters of ♀ 2994		3	10	17	19	14	5	2	1																71	12.42	.73	5.9
1/4 Lop, Matings F–K					4	9	20	41	27	25	12	10	2												150	14.41	.85	5.9
3/4 Lop, Matings A–E														2	2	1	1	3	5	9					23	19.13	1.01	5.3
3/4 Lop, Matings F–H														1		2	2	5	6	8	11	5	4	1	45	20.01	1.04	5.2

conversion factor of one mm for each hundred grams of weight. The observed ear length was increased if the animal weighed less than 2,400 grams and decreased if the animal weighed more than 2,400 grams. Thus were obtained the calculated results shown in table 5. As thus treated, the F_1 populations become less variable, and the difference between the progeny of small mothers (Matings A–E) and the progeny of large mothers (Matings F–K) becomes less. The former group still has the greater C.V. (5.5 as compared with 3.7), but this difference is due, principally, to the very aberrant individual produced in Mating A which had an actual ear length of 11.6 cm and a calculated ear length of 14.0 cm (table 5). Nevertheless, the mean and mode for the ear length of the F_1 progeny of small mothers remain lower than those for the F_1 progeny of larger mothers (Matings F–K). They differ by almost two centimeters. This indicates that the two groups differ by more than body size alone. The small mothers apparently carry certain genes which tend to make the ears short, irrespective of body size. But it may be that this apparent difference is due, in part at least, to the known earlier maturity of rabbits of racially small body size. The earlier the maturity of a rabbit, the more nearly would it have completed its growth at four months of age. The amount by which it falls below standard size would thus be *underestimated*, and the added ear length computed would fall below what it would be if all individuals had, at four months old, completed the same proportion of their full growth.

The F_2 populations show a relation similar to that shown by the F_1 populations. Those from small and short-eared mothers (Matings A–E) have ears over a centimeter shorter than those derived from Matings F–K. Both groups are more variable than the F_1 groups from which they were derived. The C.V. for the F_1 groups were 7.0 and 4.4 respectively; for the corresponding F_2 groups the values are 8.8 and 6.6 respectively. Note, however, that the variation in F_2, as well as in F_1, is slightly less when corrected or "equivalent" values are taken (table 5) than when uncorrected values are taken, as in table 4.

The 1/4 blood lop populations produced by backcrossing F_1 to the short-eared parent races, as calculated in table 5, cover the range between the ear length of ordinary rabbits (non lops) and that of the F_1 populations. There is still the same difference between the derivatives of Matings A–E and the derivatives of Matings F–K, the former being shorter in ear length by two centimeters, either because of the presence of special genes for short ear in the small races, or because their earlier maturity results in under correction, or for both reasons. The variability (C.V.) is the same in both groups of 1/4 blood lops (5.9). It is less than in the F_2 groups, but greater than in the F_1 groups, as we should expect on the multiple factor hypothesis.

Similar differences between the derivatives of Matings A–E and the derivatives of Matings F–K are found among the 3/4 blood lops (last two rows of table 5). The former are shorter eared by almost one centimeter. Their variability (C. V.) is substantially the same, but is somewhat less than that of the 1/4 blood lop populations. This again is what we should expect, as the lop-eared male which sired all the 3/4 blood lop individuals was purer, racially, than the mothers of the ½ blood lop populations. His race had been long selected for maximum ear length. The mothers in matings A-K and in the backcrosses which produced the ½ blood lop populations, were from races largely unselected as regards ear length. The equivalent ear lengths of the 3/4 blood lops do not quite cover the range between F_1 and the pure lop-eared ancestor.

On the whole, there is nothing in the results of these experiments at variance with the accepted multiple factor theory of inheritance, which supposes that an apparently blending inheritance results from the action of numerous genes located in many different chromosomes and so segregating independently.

However, we can not ignore the possibility that cytoplasmic, as well as gene, influences may be involved in producing an altered ratio between body weight and ear length, as, for example, in the peculiar short-eared F_1 female, 2994. She is similar in body size to her sibs, and yet has much shorter ears. It would seem, accordingly, that she must possess one or more genes which inhibit the action of the lop-ear genes inherited from her sire. If so, she should be more highly heterozygous for genes affecting ear length than her more normal sisters. For, like them, she would have inherited from their common sire (the purebred lop male) a full set of lop-ear genes, but from their mother she would have inherited *more* than their equipment of short-ear genes. Hence she would be more highly heterozygous than her sisters, and her gametic output would be potentially more variable. In reality, however, when mated to a purebred Himalayan male, she produced a group of 18 offspring which were *less variable* than the 71 offspring produced by her sisters mated to the same male.

A possible alternative explanation, in terms of genes, would be to suppose that a mutation had occurred in the gamete contributed by the lop-eared male to produce ♀ 2994, so that it carried less than the normal equipment of lop-ear genes. The zygote, in that case, (1) would have a lower (somatic) ear length than her sibs of identical parentage, and (2) would be less heterozygous than they for genes affecting ear length, as is indicated by the 1/4 lop population (table 4).

A more orthodox Mendelian explanation is equally possible, it being assumed that an extreme minus variant coming from the lop-eared parent happened to combine with an extreme minus variant from the short-eared parent, the result being a zygote somatically extremely low in ear length but with only normal variability in the next generation about a lowered (intermediate) mean.

The blending or intermediate character of the inheritance of lop-ears, both in F_1 and in the backcrosses, shows that the genes which differentiate pure lop-eared rabbits from ordinary ones must be numerous and cumulative in their action. How numerous they are, it is impossible to say. Application of the CASTLE-WRIGHT formula (CASTLE 1921) to the F–K matings (table 6) indicates that the lop race differs from the F–K non-lop parental animals by about 22 genes for ear size, if it be assumed that each of these genes exercises the same quantitative influence as every other such gene. Since this assumption is of doubtful validity, the estimated number, 22, is probably too high.

A study of the range of variability of F_2 and backcross generations often allows one to estimate the number of important genes affecting the expression of a quantitative character. Possibly both parental types (short and lop-ears) would have been regained in the F_2, had a sufficiently large generation been raised. In the backcross to the parental race with short ears, 20 out of 150 of the backcross young had ears as short as those of the short-eared parents (table 4). The parental range was also regained when the F_1 animals were backcrossed to the lop race. Were there as many as 10 factor differences (all equal and additive in their action) between short- and lop-ears, we should expect to regain the parental type of factor combination in only one out of 1,024 animals. Actually we regained the parental type in about one out of every 10–50 animals, which allows us to conclude that the number of important factor pairs differentiating the lop race from ordinary rabbits is probably less than ten.

If genes located in particular chromosomes are responsible for the greater ear size of lop-eared rabbits, the question arises, is it possible to identify such chromosomes by means of linkage studies? We have a certain amount of data bearing on this question, but its indications are wholly negative.

1. In backcrosses to the short-eared Himalayan race employed in Mating A, there were produced 48 colored young having a mean ear length of 14.91 cm; also 34 Himalayans having a mean ear length of 14.60 cm, a non-significant difference indicating no linkage between short ears and albinism.

2. The dominant English coat pattern was introduced into certain of the F_1 individuals in Mating H. Among the 1/4 lop animals derived from this cross there were 79 English individuals having an estimated ear length of 16.78 cm, and 51 non-English with an average ear length of 16.62 cm, an insignificant difference.

3. The agouti factor was introduced into certain F_1 individuals by the non-lop parent. Among the 1/4 lop anmials derived from Matings F–K there were 34 agouti individuals with an average ear length of 16.86 cm, and 24 non-agoutis with an ear length of 16.73 cm, a difference devoid of significance.

4. The lop-eared male was a sooty yellow. All F_1 individuals which were black pigmented consequently carried yellow as a recessive character which should show coupling with lop-ear. Back-crosses of such F_1 black females with their father produced, among the 3/4 lops, 29 sooty yellows and 32 blacks. The former had an average ear length of 19.63 cm; the blacks had an ear length of 20.04 cm. There is thus seen to be no difference in ear length favoring the yellow young.

These findings, inconclusive though they are, on account of the small numbers observed, give no indication of the presence of genes for lop-ear in the four chromosomes which harbor the genes for albinism, English, agouti, and yellow, respectively. It is true that these are only four of the 22 chromosome pairs of the rabbit, and it is possible that genes affecting ear length are located in the 18 other chromosome pairs. It is also true that if a single gene for lop-ear were present in one of these chromosomes, yet had by itself only a minor influence on ear length, this influence might well be masked by the influence of other genes present in undetermined combinations.

We have seen that ear size is influenced by general body size, which, in turn, is influenced by rate of development of the fertilized egg. But there must be also in lop-eared rabbits an especial accelerated development in the head region, which makes the ears of lop-eared rabbits, or lop-eared crossbred derivatives, noticeably larger at birth than the ears of ordinary rabbits. The persistence of such an initial difference would account satisfactorily for the subsequent greater ear length of lop-eared rabbits. We can not assume that the supposed accelerated development in the head region is due to a special localized organization of the cytoplasm of the egg, since we know that the sperm is equally capable of inducing it.

The case of the short-eared mouse shows us that a single gene may be responsible for retarded development of the ear, which makes its ultimate length less. We know definitely that the gene in this case is located in the same chromosome with the gene for dilution. It seems reasonable to suppose that, in the case of the rabbit, a series of gene mutations having an opposite effect on ear length has taken place; that each of these, considered separately, has only a minor effect, but cumulatively they make all the difference between the lop-eared and ordinary rabbit. Yet the initial mutation or chance coincidence of mutations which formed the starting point for human selection must have been sufficiently obvious to attract attention, after which new mutations having a like effect would be incorporated and retained in the selected race. There is reason to think that mutations having a contrary effect also occur among rabbits as well as among mice. The breeders' standard for Polish rabbits insists that the ears be as short as possible. This shortness is secured, in part, by keeping the body small, but to some extent also by special shortening genes. For it will be observed, in tables 4 and 5, that in our Mating A–E, in which mothers of small racial size were used, the progeny have shorter ears than the derivatives of mothers of larger racial size, even when correction is made for the differences in body size among the progeny. This indicates that in the rabbit, although there is (other things being equal) a strong correlation between general body size and ear size, there are also special genes influencing ear size, and chance mutations in these have been utilized in the production of lop-eared rabbits in large-bodied races and of notably short-eared rabbits in small-bodied races. This conclusion is in harmony with WRIGHT's (1932) finding, based on a statistical examination of CASTLE's size inheritance data, that although the various parts of the body form a harmonious system varying in unison and controlled by general body size, nevertheless to a minor extent variation in one part takes place independently of other parts.

SUMMARY

1. A renewed study of the inheritance of ear length in crosses between lop-eared and ordinary short-eared rabbits supports the multiple factor interpretation first applied to the case by LANG.

2. Ear length among ordinary breeds of rabbits is closely correlated with general body size. A similar influence of body size on ear length is found also among lop-eared rabbits, but special mutated genes in the case of purebred lop-eared rabbits nearly double the ear length as compared with that of ordinary rabbits of like body size.

3. The increased size of the ears in lop-eared rabbits has not been attended by any change in the shape of the ear. The ratio of ear length to ear width is nearly the same in all breeds of rabbits and at all ages between one month and maturity. It varies closely about a modal value of 0.56.

4. There are indications that mutations decreasing the size of the ears in relation to that of the body have also occurred among rabbits, and that such mutations have been incorporated in such small-bodied and short-eared breeds as Polish.

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STUDIES OF A SIZE CROSS IN MICE, II

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SEVERAL years ago GREEN made a cross between two races of mice of very different body size, the experiment being designed to throw light on the mechanism of size inheritance. His working hypothesis was that differences in body size are determined by genes borne in chromosomes, that such genes are numerous, and that accordingly it should be possible to demonstrate the existence of certain of them in the same chromosomes as contain coat color genes.

As a small parent race, he used *Mus bactrianus*; as a large race of nearly twice as great body size, he used LITTLE's dilute brown race of *Mus musculus*. The small race contained the three independently inherited dominant genes, agouti (A^w), black, and dilution. The large race contained the three recessive alleles, non-agouti, brown, and dilution. In F_2 and backcross populations it was found that larger body size was associated with each of the recessive segregates, non-agouti individuals being larger than agoutis, browns larger than blacks, and dilutes larger than intense individuals. This was regarded as supporting the hypothesis that in each of the three marked chromosomes derived from the larger parent there was present one or more genes for larger body size. On further investigation, however, GREEN concluded that the differences observed were sufficient to have unmistakable statistical significance only in the case of the chromosome containing the brown gene. This was the state of the question when we undertook its further investigation. We attempted a substantial repetition of GREEN's experiment with slightly different stocks available to us. As a small parent race we used at first not *Mus bactrianus* but a supposed domestic derivative of it, the race of black-and-white Japanese waltzing mouse studied by GATES (1926). Later, through the kindness of Drs. GREEN and LITTLE, we were able to employ also the same strain of *Mus bactrianus* which GREEN had employed. As the large parent race in both these crosses (which, for convenience, we may call Cross 1 and Cross 2, respectively), we used a derivative of LITTLE's dilute brown race in which two additional recessive gene mutations had been incorporated, namely pink-eye and short-ear.

The F_1 animals produced by both crosses were only a little smaller than animals of the larger parent race but were of remarkable vigor, fecundity, and longevity. Both were reciprocally backcrossed to LITTLE's dilute brown race of *M. musculus.* The F_1 animals from Cross 1 were also backcrossed to the actual *musculus* parent race, the pink-eyed short-eared dilute brown race of GATES. We may now proceed to the discussion of these backcross populations.

BACKCROSS OF F_1 FEMALES FROM CROSS 1 TO D BR MALES

In a previous paper, we have discussed the mature body weight and the body length at age six months of this population consisting of 1,236 animals. We have shown, in confirmation of GREEN'S earlier conclusion, that brown individuals are heavier and longer bodied than blacks, and that dilute individuals are heavier and longer-bodied than intense ones, though the difference is less in the case of dilution than in that of brown, as GREEN had previously observed. But we have been led to adopt an explanation for the manifest superior size of brown and of dilute segregates different from that of GREEN. Instead of invoking the action of size genes located in the same chromosomes as the brown and dilution genes, we assume that it is the physiological action on growth of the brown gene and the dilution gene themselves which produces the observed effects. To this question we shall return later. For a detailed statement of results as regards weight and body-length, the reader is referred to our previous publication. CASTLE, GATES and REED (1936).

At the time these mice were chloroformed and measured as to body length, their tail length was also measured by SUMNER'S method, keeping the body under a uniform tension of 20 grams. We have only recently studied the data on tail length, which show one surprising and unexpected feature. The greater size of brown and of dilute individuals, as compared with black and intense individuals respectively, finds expression as expected in all three criteria studied, namely, (1) maximum weight at or prior to six months of age, (2) body length, and (3) tail length at age six months. Brown has regularly a greater influence than dilution on weight and body-length, as reported in our previous publication, but as regards tail-length their relations are reversed. Dilution has a greater influence than brown in elongating the tail. This is the unexpected feature of a study of tail length in the backcross population, and it finds support, as we shall see, in the backcross from Cross 2. The data on the variation in tail length of the backcross population are contained in table 1. The numbers there reported (637 females and 439 males) are smaller than for the body length studies reported in our previous paper because of occasional injury to the tail (particularly in the case of males caged together). A single domineering male will, by biting the tails of his cage mates, destroy the possibility of obtaining normal tail measurements for them. Nevertheless the available data for both sexes are entirely in harmony, in that they show that the tail length of blues is greater than that of browns, the order of size among the color classes as regards tail length being (1) black, (2) brown, (3) blue, and (4) dilute brown. Brown females as compared with blacks have an increased tail length of 1.5 percent, but dilute females as compared with intense have tails 2.3 percent longer. Also brown males as compared with blacks, have tails 1.2 percent longer, but dilute males as compared with intense have tails 2.6 percent longer. In both sexes dilution has an influence on tail length superior to that of brown, although in regard to body length and total weight the relation is reversed. Both mutant genes (brown and dilution) for which segregation is occurring in this backcross population have a positive (increasing) effect on general body size, as indicated in body weight, body length, and tail length. Their combined action is also additive or cumulative in all cases. But apart from general body size, there would seem to be also a special specific influence of dilution on tail length.

TABLE 1

Variation in tail length of four different color classes from matings of F_1 females from Cross 1 to d br males

	FEMALES			MALES		
	NO.	MEAN	S.D.	NO.	MEAN	S. D.
Black	151	75.5±.22	3.98±.15	103	78.7±.27	4.09±.19
Blue	170	77.5±.19	3.76±.14	116	80.1±.24	3.86±.17
Brown	159	76.9±.22	4.08±.15	121	79.0±.27	4.40±.19
Dil. Brown	157	78.5±.22	4.07±.15	99	81.7±.23	3.38±.17
Total	**637**	**77.2±.11**	**4.10±.08**	**439**	**79.8±.13**	**4.15±.09**
All Blacks	321	76.5±.15	3.96±.10	219	79.4±.18	4.03±.13
All Browns	316	77.7±.16	4.18±.12	220	80.2±.19	4.20±.13
All Intense	310	76.2±.15	4.07±.12	224	78.8±.18	4.10±.13
All Dilute	327	78.0±.15	3.93±.10	215	80.84±.17	3.74±.12
Brown minus Black		1.20±.22=5.4			.80±.26=3.0	
Dilute minus Intense		1.77±.21=8.4			1.98±.24=8.2	

BACKCROSS OF F_1 MALES FROM CROSS 1 TO D BR FEMALES

We were able to obtain a smaller population from this backcross than from the reciprocal one already discussed because of the smaller number of mothers available. The number studied is approximately 100 of either sex, more exactly 99 females and 106 males. Comparative data on the segregation in this and the reciprocal backcross are contained in table 2.

In making this comparison we are interested in two questions: (1) will the chromosomes marked by brown and dilution here show the same accelerating influence on growth as in the reciprocal backcross, and (2) is there a significant difference in size between the progeny of F_1 mothers

TABLE 2

Comparison of the several classes of individuals produced by reciprocal backcrosses between F_1 animals and the "d br" race

	FROM ♀F₁×♂*d br*				FROM ♀*d br*×♂F₁				DIFFERENCE		
FEMALES	NO.	MEAN WT. GMS.	MEAN BODY MM.	MEAN TAIL MM.	NO.	MEAN WT. GMS.	MEAN BODY MM.	MEAN TAIL MM.	WEIGHT GMS.	BODY MM.	TAIL MM.
Black	337	21.91±.08	91.51±.12	76.5 ±.15	41	23.18±.29	93.51±.34	76.82±.51	1.27±.30	2.00±.36	.32±.53
Brown	328	22.61±.12	92.84±.12	77.7 ±.16	58	23.73±.25	94.71±.27	77.27±.36	1.12±.27	1.87±.29	−.53±.39
Intense	324	22.04±.09	91.79±.12	76.2 ±.15	49	22.75±.24	92.88±.23	75.72±.44	.71±.25	1.09±.26	−.48±.46
Dilute	351	22.45±.10	92.55±.12	78.0 ±.15	50	24.24±.28	95.52±.30	78.27±.39	1.79±.29	2.97±.32	.27±.41
Totals and means	**675**	**22.24±.10**	**92.17±.12**	**77.1 ±.15**	**99**	**23.93±.25**	**94.18±.25**	**77.0 ±.40**	**1.69±.27**	**2.01±.28**	**−.01±.42**
MALES											
Black	280	28.34±.13	95.77±.14	79.4 ±.18	62	28.28±.21	97.14±.22	78.85±.39	.15±.24	1.37±.27	−.55±.43
Brown	291	29.56±.12	97.73±.10	80.2 ±.19	44	29.63±.29	98.48±.29	79.71±.51	.07±.27	.65±.30	−.49±.54
Intense	278	28.67±.12	96.26±.14	78.8 ±.18	53	28.51±.25	97.21±.24	77.58±.47	−.49±.27	.95±.27	−.22±.50
Dilute	293	29.34±.13	97.21±.14	80.84±.17	53	29.17±.23	98.24±.27	80.64±.32	−.17±.26	1.03±.30	−.20±.36
Totals and means	**571**	**29.00±.12**	**96.74±.13**	**79.82±.17**	**106**	**28.95±.24**	**97.76±.25**	**79.25±.35**	**−.05±.26**	**1.02±.28**	**−.47±.39**

mated with d br males and the progeny of d br females mated with F₁ males? In other words, has the large race mother any superior influence on the size of her offspring? Table 2 contains the answer to the first question, an emphatic affirmative. Brown and dilution are found in heavier and longer-bodied individuals than their alleles in this as in the reciprocal backcross. Also brown has a greater influence than dilution in both sexes in increasing the average weight and body length; but brown has in both sexes *less* influence than dilution in increasing length of the tail, exactly as in the reciprocal backcross. We may accordingly, for qualitative effects, combine the data from both backcrosses, weighting each in proportion to the number of individuals which it contains. This procedure gives us the weighted means printed in italics in table 3.

TABLE 3

Percentage change in body size effected by the gene mutations brown (b) and dilution (d) in reciprocal backcrosses between F_1 hybrids and the d br race

		FEMALES			MALES		
GENE	MOTHER	WEIGHT	BODY LENGTH	TAIL LENGTH	WEIGHT	BODY LENGTH	TAIL LENGTH
b	♀F_1	3.18	1.45	1.56	4.30	2.04	1.20
b	♀d br	2.37	1.28	.58	4.77	1.38	1.09
Weighted mean		*3.07*	*1.40*	*1.43*	*4.37*	*1.90*	*1.18*
d	♀F_1	1.86	.82	2.36	2.30	.98	2.60
d	♀d br	6.54	2.84	3.36	2.31	1.06	3.94
Weighted mean		*2.47*	*1.08*	*2.48*	*2.30*	*.99*	*2.80*

The second question, one of considerable theoretical interest, as it involves a possible differential maternal influence on the size of offspring, finds its answer in table 2. The d br (large race) mothers produce larger-bodied offspring in both sexes, if we rely on the criterion of body length alone, undoubtedly the most reliable criterion. The differences between the means of the two groups are statistically significant. If we judge by body weight, female offspring of the large race mothers are also significantly larger, but there is no significant difference in the case of males. As regards tail length, no significant difference is found in either sex.

RECIPROCAL BACKCROSSES OF F₁ ANIMALS FROM CROSS 2 TO THE D BR RACE

The F₁ animals from this cross showed (but were heterozygous for) the three dominant characters, agouti, black, and intensity. On backcrossing them with the d br race which is homozygous for the corresponding recessive alleles, non-agouti, brown, and dilution, eight classes of colored offspring were obtained. In the left half of table 4 and of table 5 will be found a summary of the data on the body size of each of these eight classes

TABLE 4

Comparison of the several classes of females produced by reciprocal backcrosses between F_1 individuals from Cross 2 and the d br race

FEMALES	FROM ♀ F_1×♂ *d br*				FROM ♀ *d br*×F_1				DIFFERENCE		
	NO.	MEAN WEIGHT	MEAN BODY	MEAN TAIL	NO.	MEAN WEIGHT	MEAN BODY	MEAN TAIL	WEIGHT	BODY	TAIL
Black Agouti	56	22.99	92.12	73.55	20	23.37	93.40	72.65	.38	1.28	−.90
Blue Agouti	62	23.48	92.67	74.47	13	24.01	94.30	75.40	.53	1.63	.93
Brown Agouti	60	23.97	93.76	75.42	16	25.55	95.06	75.33	1.58	1.24	−.09
Dil. Br. Agouti	45	24.42	94.72	77.17	17	24.82	94.94	75.79	.40	.22	−1.38
Black	56	23.30	92.75	74.41	16	24.05	94.50	76.35	.75	1.75	1.94
Blue	62	23.74	93.66	76.65	15	25.33	94.33	76.25	1.59	.67	−.40
Brown	54	24.75	93.37	74.44	15	26.28	95.00	74.59	1.53	1.63	.15
Dil. Br.	47	24.52	94.51	77.25	12	25.42	94.09	77.12	.90	−.42	−.13
Totals and means	442	23.91 ± .09	93.38 ± .10	75.42 ± .13	124	24.85	94.45	75.30 ± .26	.96	1.01	.01

TABLE 5

Comparison of the several classes of males produced by reciprocal backcrosses between F_1 individuals from Cross 2 and the d br race

MALES	FROM ♀ F_1×♂ *d br*				FROM ♀ *d br*×♂ F_1				DIFFERENCE		
	NO.	MEAN WEIGHT	MEAN BODY	MEAN TAIL	NO.	MEAN WEIGHT	MEAN BODY	MEAN TAIL	WEIGHT	BODY	TAIL
Black Agouti	56	28.79	96.24	75.69	19	29.06	97.10	74.87	.27	.86	−.82
Blue Agouti	52	29.80	98.18	78.25	14	31.92	99.71	78.82	2.12	1.53	.57
Brown Agouti	63	31.27	99.45	76.59	22	32.48	99.05	78.11	1.21	−.40	1.52
Dil. Br. Agouti	61	30.76	98.52	79.19	16	32.86	98.68	79.85	2.10	.16	.66
Black	54	29.31	96.00	75.37	16	29.78	97.18	75.83	.37	1.18	.46
Blue	51	30.48	97.74	76.98	15	31.12	99.06	78.57	.64	1.32	1.59
Brown	51	30.87	98.34	77.63	12	32.54	98.41	77.41	1.67	.07	−.22
Dil. Br.	55	30.47	97.93	78.63	19	32.50	100.03	79.72	2.03	2.10	1.09
Totals and means	443	30.40 ± .11	97.83 ± .11	77.27 ± .15	133	31.53	98.65	77.78 ± .30	1.31	.85	.51 ± .33

as indicated by weight, body length, and tail length, when an F_1 female was the mother. In the right half of these same tables will be found corresponding data for the reciprocal backcross in which a d br female was the mother.

Table 6

Percentage change in the body size of females effected by the gene mutations, a, b, and d, in reciprocal backcrosses between F_1 individuals from Cross 2 and the d br race

GENE	MOTHER	WEIGHT		BODY LENGTH		TAIL LENGTH	
		DIFF. AND P.E.	PERCENT INCREASE	DIFF. AND P.E.	PERCENT INCREASE	DIFF. AND P.E.	PERCENT INCREASE
a	♀F_1 Bact.	.46 ± .14	1.94	.21 ± .20	.33	.68 ± .28	.84
a	♀d br	.83	3.40	.05	.05	1.38 ± .52	1.84
Weighted mean		*.54*	*2.26*	*.17*	*.26*	*.83*	*1.06*
b	♀F_1 Bact.	1.40 ± .18	1.94	1.21 ± .20	1.30	.68 ± .28	.84
b	♀d br	1.33	5.48	.64	.68	.71 ± .52	.94
Weighted mean		*1.38*	*2.71*	*1.08*	*1.16*	*.68*	*.85*
d		.13 ± .17	.54	.77 ± .20	.82	1.77 ± .25	2.37
d	♀d br	.08	.33	−.07	−.07	1.52 ± .48	2.04
Weighted mean		*.12*	*.49*	*.58*	*.62*	*1.71*	*2.29*

Table 7

Percentage change in the body size of males effected by the gene mutations, a, b, and d, in reciprocal backcrosses between F_1 inidividuals from Cross 2 and the d br race

GENE	MOTHER	WEIGHT		BODY LENGTH		TAIL LENGTH	
		DIFF. AND P.E.	PERCENT INCREASE	DIFF. AND P.E.	PERCENT INCREASE	DIFF. AND P.E.	PERCENT INCREASE
a	♀F_1 Bact.	−.29 ± .21	−1.40	−.81 ± .22	−.83	−.23 ± .29	−.30
a	♀d br	−.14	−.44	.03	.03	.59 ± .56	.76
Weighted mean		*−.25*	*−1.18*	*−.60*	*−.61*	*−.04*	*−.05*
b	♀F_1 Bact.	1.30 ± .21	4.40	1.56 ± .20	1.60	1.50 ± .30	1.96
b	♀d br	2.12	6.97	.78	.79	2.14 ± .58	2.79
Weighted mean		*1.49*	*4.99*	*1.37*	*1.41*	*1.64*	*2.13*
d	♀F_1 Bact.	.32 ± .22	1.00	.74 ± .22	.75	2.10 ± .30	2.75
d	♀d br	1.13	3.66	1.43	1.46	3.03 ± .57	3.97
Weighted mean		*.51*	*1.61*	*.89*	*.91*	*2.31*	*3.03*

The question as to the relative influence of F_1 mothers and d br mothers on the size of their offspring will be clear from an examination of these tables. In each of the eight color classes, in both sexes, the d br mothers produce the heavier offspring. The same superiority of the progeny of d br mothers is found as regards body length in seven of the eight color classes of each sex, and of course emphatically when all color classes are combined.

As regards tail length, there is no uniform superiority of the progeny of large race mothers, though in general, particularly among the males, d br mothers produce the longer-tailed progeny.

The relative influence of each of the recessive genes on the body size of the offspring in these backcrosses is shown in tables 6 and 7. Non-agouti females are larger than agouti females by all three criteria, weight, body length, and tail length (table 6), though as regards body length (the best criterion) the difference is negligible. But among the males a contrary relation is found, since agouti individuals are larger-bodied than non-agoutis by all three criteria. It is accordingly not shown that the non-agouti mutation, though derived from the larger parent race, has any consistent influence either to increase or to decrease body size.

The brown mutation (*b*) is shown in this cross, as in all others, to increase body size, as judged by all three criteria. Brown females are more than 2.5 percent heavier and brown males are 5 percent heavier than black individuals of the same sex. Brown females are also 1.1 percent longer bodied and brown males are 1.4 percent longer-bodied than black ones. Brown females have tails less than 1 percent longer than blacks, but brown males have tails more than 2 percent longer than blacks. By all criteria males show a greater increase in size than females, as in the backcrosses previously discussed.

Dilution, as in the other crosses, increases size in both sexes in lesser amount than brown as regards weight and body length, but in greater amount as regards tail length. Tail length is increased 2.29 percent in dilute females and 3.03 percent in dilute males, the corresponding increases for brown individuals being .85 percent and 2.13 percent respectively. A special influence of the dilute gene (or the dilute chromosome) on tail length is thus shown to occur in four backcross populations, namely, in the reciprocal backcrosses both from Cross 1 and from Cross 2.

BACKCROSS OF F_1 FEMALES FROM CROSS 1 TO MALES OF THE MATERNAL (SE) RACE

This backcross, like those already described, was made reciprocally, but owing to an insufficient supply of se females being available, only the backcross in which F_1 mothers were used has as yet produced a population large enough for useful discussion. We shall accordingly, for the present, confine our attention to this alone.

This backcross is of interest not only because it gives additional evidence on the effect of brown and dilution on body size but also because it yields data on the effects of two additional gene mutations, pink-eye and short-ear. Since short-ear is closely linked with dilution, the number of phenotypes produced by the backcross is eight, except when a crossover occurs (about once in a thousand). The number of individuals produced was larger in this than in any of our other backcrosses. We present data on a population of 930 females and 917 males, a total of 1,847 individuals. It would be a needless use of space to report the detailed study which has been made of the variation of each color class in weight, body length, and tail length. We shall content ourselves, therefore, with a summary of the data. Tables 8 and 9 show, for each sex separately, the mean values and variability as regards the size characters studied in the case of each color class. Table 10 shows the influence of each mutant gene on the size characters studied. In the case of short-ear and dilution, it is possible to estimate only their combined effect. In other backcrosses we have found that dilution regularly increases body size, but it is clear from this cross that short-ear decreases size more than dilution increases it, so that their combined action is a decrease even greater than the increase made by brown, which, up to this time, had been found more influential than any other single gene mutation on body size. The decrease in weight amounts to 3.67 percent for females, 5.74 percent for males. How great the decreasing effect of short-ear on body size would be, apart from dilution, it is impossible at present to state. Among other gene mutations which decrease body size must

TABLE 8

Comparative variability of females of the several color classes produced by a backcross of F_1 females to the se race

	WEIGHT			BODY LENGTH		TAIL LENGTH	
	NO.	MEAN	S.D.	MEAN	S.D	MEAN	S D.
Black	126	23.12 ± .18	2.95 ± .12	91.00 ± .20	3.25 ± .14	79.20 ± .26	4.13 ± .18
Blue se	133	22.82 ± .15	2.66 ± .11	90.27 ± .16	2.73 ± .11	78.77 ± .23	3.96 ± .16
Brown	124	22.71 ± .16	2.65 ± .11	90.59 ± .20	3.30 ± .13	79.32 ± .28	4.62 ± .10
D br se	124	22.49 ± .17	2.77 ± .12	90.92 ± .19	3.20 ± .14	78.29 ± .27	4.55 ± .20
PE Black	118	23.21 ± .17	2.81 ± .12	90.32 ± .20	3.21 ± .14	78.12 ± .25	3.90 ± .17
PE Blue se	98	22.02 ± .16	2.44 ± .12	89.03 ± .25	3.71 ± .18	78.13 ± .29	4.22 ± .20
PE Brown	119	24.09 ± .20	3.31 ± .14	91.42 ± .20	3.34 ± .15	78.91 ± .23	3.84 ± .16
PE d br se	88	22.50 ± .10	2.65 ± .13	90.76 ± .19	2.99 ± .15	78.03 ± .30	4.18 ± .21
Totals and means	**930**	**22.83 ± .06**	**2.78 ± .04**	**90.65 ± .07**	**3.21 ± .05**	**78.75 ± .09**	**4.14 ± .06**

TABLE 9

Comparative variability of males of the several color classes produced by a backcross of F_1 females to the se race

	WEIGHT			BODY LENGTH		TAIL LENGTH	
	NO.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
Black	126	28.11 ± .19	3.20 ± .13	94.32 ± .20	3.24 ± .14	80.72 ± .28	4.50 ± .20
Blue se	117	27.81 ± .19	3.04 ± .13	94.36 ± .17	2.64 ± .12	80.75 ± .29	4.40 ± .20
Brown	101	30.31 ± .25	3.81 ± .18	96.41 ± .24	3.61 ± .17	82.74 ± .35	5.11 ± .24
D br se	117	28.46 ± .21	3.47 ± .15	95.33 ± .22	3.60 ± .16	81.06 ± .29	4.52 ± .20
PE Black	100	28.42 ± .22	3.24 ± .15	94.51 ± .23	3.40 ± .16	80.49 ± .33	4.70 ± .23
PE Blue se	121	26.69 ± .18	2.98 ± .13	93.12 ± .19	3.09 ± .13	79.85 ± .29	4.65 ± .21
PE Brown	136	29.46 ± .18	3.20 ± .13	95.83 ± .19	3.39 ± .13	81.53 ± .29	4.84 ± .20
PE d br se	99	27.02 ± .25	3.68 ± .18	94.42 ± .19	2.79 ± .13	81.16 ± .25	3.53 ± .18
Totals and means	**917**	**28.38 ± .08**	**3.60 ± .06**	**94.80 ± .07**	**3.37 ± .05**	**81.01 ± .10**	**4.60 ± .07**

undoubtedly be included dwarf and waltzing, but as yet we have no data on their quantitative influence.

Brown, as in other backcrosses, has a tendency to increase all size characters studied. Pink-eye, on the other hand, though also derived from the larger parent race in the original cross, has a tendency to decrease size in the backcross. This tendency is manifested in all characters studied except weight in the case of females. Here some extremely fat individuals made the average weight for all pink-eyed females exceed that of the dark-eyed, but as regards body-length and tail-length, pink-eyed females as well as males, fell below the dark-eyed. The effect of pink-eye in decreasing body size is small but consistent in both sexes (except for weight in females) and so may be accepted as genuine.

TABLE 10

Influence of particular genes on body size in the backcross to the se race, as indicated by percentage increase or decrease (—) of the average.

	FEMALES			MALES		
	WEIGHT	BODY	TAIL	WEIGHT	BODY	TAIL
Brown	.68	.60	.10	3.80	1.53	1.45
SE+Dilution	−3.67	−.40	−.74	−5.74	−1.01	−.82
Pink-eye	.74	−.09	−.76	−2.77	−.19	−.69

We are now in a position to consider the theoretical question, is it linkage with size genes borne in the same chromosomes, or is it the physiological action on growth of the mutant genes themselves which is responsible for the effects noted in these mouse crosses? The larger parent introduced mutant genes which mark four of the twenty chromosomes of the mouse, namely, (1) agouti, (2) brown, (3) dilution and short-ear, and (4) pink-eye. On the linkage hypothesis we should expect to find larger body size associated with each of these genes in the backcross segregates. From the results of our study it appears that there are no linked size genes in the agouti chromosome or the pink-eye chromosome, since agouti individuals do not consistently differ from non-agoutis, and pink-eyed segregates are actually smaller than dark-eyed, contrary to expectation based on the linkage hypothesis. Dilution is associated with larger size when short-ear is not present, and brown is regularly found in the larger-bodied segregates. For the linkage hypothesis we have then, thus far, two positive and two negative tests. But under further examination of the positive cases, the linkage hypothesis breaks down. The case of dilution and short-ear affords a crucial test. Dilution by itself increases size, but when short-ear is present, size is decreased. But dilution and short-ear are closely linked, crossovers occurring less than once in a thousand times. If linkage with a size gene were responsible for the larger size of dilute animals, this gene should become effective irrespective of the presence of short-ear, since it would become homozygous when *either* dilution or short-ear became homozygous; but we find that dilution increases body size even when short-ear is present as a heterozygote; yet when short-ear is homozygous, size is decreased even in the presence of dilution. We are thus forced to assume a physiological action of short-ear on growth. If we concede the existence of such an effect to short-ear, there is no reason why we should not concede also to dilution an influence of a contrary character. Indeed that seems to be the only logical explanation of the observed facts. But if short-ear and dilution affect growth by their physiological action and thus influence body size, there is every reason to suppose that similar action is exercised by brown. FELDMAN (1935) has presented evidence supporting this view.

Size inheritance has long been supposed to result from the joint action of many genes, but in mammals we have hitherto lacked evidence of what these genes were or how they acted. For the mouse we are now able definitely to identify as genetic modifiers of body size the mutant genes brown, dilution, and short-ear. They act through their influence on general body size as manifested in length of trunk and tail and total body weight. This means probably that their action begins early and continues throughout growth. For dilution we have the interesting observation that besides its general action on body size, it exercises a special influence toward increase in the length of the tail, not great enough, however, to counteract the contrary influence of short-ear when present. WRIGHT, DAVENPORT, SUMNER, and others have found evidence of the existence in mammals of special genetic influences local in their action. This demonstrated action of dilution supports that interpretation. It must mean, in physiological terms, that though the dilution gene is probably active throughout growth and thus influences size of the animal as a whole, it is especially active at that stage in development when the tail is being formed.

There is a genetic difference between *Mus bactrianus* and the Japanese waltzer in length of tail. The tail in *M. bactrianus* is relatively shorter. This difference is manifested in the backcross populations. For animals of the same body size, those descended from a Japanese waltzer have longer tails than those descended from *M. bactrianus*. The backcross populations produced by d br males, when mated with F_1 females from Cross 1 and from Cross 2, may be compared as to their size indices in table 11. The animals derived from Cross 2 are seen to be about 1 percent longer-bodied and 10 percent heavier, but their tails are 3 or 4 percent shorter. This is independent of the action of dilution in lengthening the tail, because its influence is present equally in both populations, and its action is positive in both cases, as already stated. But the genetic basis on which dilution may act is different in the two backcrosses. *M. bactrianus* has a genetic complex for shorter tail length than the Japanese waltzing mouse.

TABLE 11

Mean size of backcross animals from Cross 1 and Cross 2 compared

	WEIGHT	BODY LENGTH	TAIL LENGTH
Females, from Cross 1	22.25	92.17	77.20
Females, from Cross 2	24.85	93.38	75.30
Ratio	111.6	101.3	97.5
Males from Cross 1	28.99	96.71	80.84
Males from Cross 2	31.53	97.83	77.78
Ratio	108.7	101.1	96.2

Correlation is fairly high between body length and weight; .684 ± .012 in the case of males, .642 ± .013 in the case of females in the backcross to the *se* race, our largest population. Between body length and tail length the correlation is less close, owing to the greater variability of tail length probably through environmental influences. The coefficients obtained for the same backcross populations as those already mentioned were for males .466 ± .018, and for females .406 ± .019. It is again noteworthy that higher coefficients are obtained for males than for females, indicating that growth processes are more advanced in the case of males than of females, and to a greater extent genetically determined, accidents of development and errors of observation being relatively smaller.

SUMMARY

1. Crosses were made between females of an inbred race of *Mus musculus* having pink eyes, short ears, and dilute brown non-agouti coat, mated (1) with black-and-white Japanese waltzing mice or (2) with *Mus bactrianus*.

2. Cross 1 produced dark-eyed, long-eared, intense black F_1 individuals. Cross 2 produced dark-eyed, long-eared, intense agouti F_1 individuals. In size the F_1 animals from both crosses were nearly as large as the larger parent race, and of remarkable vigor, fecundity, and longevity.

3. F_1 individuals from Cross 1 were reciprocally backcrossed to LITTLE's dilute brown race of *Mus musculus*. In both backcrosses brown individuals were of larger body size than black ones, and dilute individuals were larger than intense ones by three different criteria, namely, (a) mature body weight, (b) body length, and (c) tail length.

4. The influence of brown was found to be greater than that of dilution as judged by body weight and body length, but as regards tail length dilution was found to be more influential than brown.

5. Of the reciprocal backcrosses, that in which the pure *musculus* (d br)

race was the mother produced consistently larger offspring as regards weight, body length, and usually as regards tail length also.

6. F_1 individuals from Cross 2 were also reciprocally backcrossed to LITTLE's dilute brown race. In these backcrosses, as in those from Cross 1, brown individuals were larger than black ones, and dilute individuals were larger than intense ones by all three criteria, weight, body length, and tail length.

7. As in the backcrosses from Cross 1, so also in the backcrosses from Cross 2, brown was more influential than dilution in increasing weight and body length, but dilution was more influential than brown in increasing tail length. This leads us to the tentative conclusion that dilution has a special effect on tail length over and above its effect on general body size.

8. Again as in the backcrosses from Cross 1, so also in the backcrosses from Cross 2, the pure *musculus* race as mother produces larger-bodied offspring than the reciprocal backcross, regularly in weight and body length, and usually in tail length also. The larger racial or individual size of the mother is thus shown to be a factor in producing large body size in her offspring. Such influence, if genetic, must be extra-chromosomal, as has been shown by LITTLE as regards the inheritance of susceptibility to spontaneous cancer in reciprocal crosses and backcrosses in mice.

9. The F_1 animals from Cross 1 were also backcrossed to the maternal parent race, the pink-eyed short-eared dilute brown race of GATES. There resulted 8 color classes, segregation occurring simultaneously for three independent characters, (1) pink-eye, (2) brown, and (3) the closely linked characters, short-ear and dilution.

10. In this backcross, as in those already described, brown individuals were larger-bodied than black ones. Pink-eyed individuals were slightly smaller than dark-eyed ones, and agouti individuals showed no consistent difference, being larger-bodied in the case of females but smaller in the case of males. Dilute individuals we should have expected to be larger-bodied than intense in this as in other backcrosses, but because of its regular association with short-ear, which strongly reduces body size, the short-eared dilute classes were actually smaller-bodied than the intense long-eared classes by all three criteria employed.

11. Linkage with size genes is an inadequate explanation of the size differences found in backcross populations. Direct physiological action on growth of the mutant genes studied is the interpretation preferred by us. As regards their influence on size, brown and dilution have accelerating effects, pink-eye has a slightly retarding, and short-ear a strongly retarding effect. Agouti probably has no effect.

12. Fairly high correlation coefficients indicate that general growth

processes rather than local ones are chiefly affected by these mutant genes, but a special local action of dilution on tail length is indicated.

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CHROMOSOME COILING IN RELATION TO MEIOSIS AND CROSSING OVER

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DURING the past five years a considerable amount of evidence has been obtained which strongly supports JANSSENS' partial chiasmatypy theory of chiasma formation. This evidence includes (1) the meiotic configurations found in trivalents and quadrivalents (DARLINGTON 1930); (2) the frequency of figure-8 chromosomes in segmental interchange rings of Pisum (SANSOME 1933); (3) the relation between the failure of chiasma formation and absence of crossing over (DOBZHANSKY 1932), BEADLE 1933); (4) the relation between chiasma frequency and crossover frequency (BEADLE 1932, DARLINGTON 1934); (5) the similarity in effect of environment on chiasma frequency and crossover frequency (WHITE 1934); (6) the behavior of heteromorphic homologues (HUSKINS and SPIER 1934); (7) the evidence for interference in both chiasma formation and crossing over (HALDANE 1931); (8) the negative correlation of frequencies for both chiasmata and crossing over in bivalents of the same cell (MATHER and LAMM 1935); (9) the types of chromatid associations at meiosis (HEARNE and HUSKINS 1935); and (10) the types of interlocking between non-homologous bivalents at meiosis (HUSKINS and SMITH 1935, MATHER 1935). Some of this evidence can not be considered as critical, because its interpretation is based on unproved assumptions; but the cumulative value of the various lines of evidence does prove the validity of Janssens' theory. The direct relation between chiasma formation and crossing over certainly facilitates cytogenetic studies and the analysis of the causal factors in crossing over, a feature not found in the hypothesis suggested by the author some years ago (SAX 1930).

Recent cytological studies of chromosome structure and behavior in mitosis and meiosis have indicated the factors which are responsible for the fundamental differences in the two types of division. Although the time of chromosome duplication is a disputed question, the general features of chromosome behavior seem to be fairly well established.

The chromonemata are in the form of *minor spirals* at anaphase in mitosis. During the formation of the daughter nucleus the minor spirals begin to uncoil, but further uncoiling is inhibited as the chromosomes pass into the resting stage. At early prophase the coiled chromosomes begin to elongate and uncoil, but at the same time the two chromatids in each chromosome begin to coil independently. By the time these *relic coils* are to truncate in *Drosophila melanogaster*. It was found that the gene for truncate was transmitted through the females in the ordinary Mendelian fashion with random segregation. The males, on the other hand, bred as though they were homozygous for the gene received from their mothers, and did not transmit the gene received from their fathers. This fact indicated that in the case of one autosome pair segregation was selective, and that the autosome retained in the functional cell at the first spermatocyte division was regularly maternal in origin. It remained to be determined how far this behavior was characteristic of the other chromosomes of this species.

A second chromosome pair was identified when two recessive characters, swollen and narrow, (METZ and ULLIAN 1929, METZ and SCHMUCK 1931) were found to show typical sex-linked inheritance. Later a dominant character, Wavy, (METZ and SMITH 1931) was secured, and more recently, two additional recessive characters, round and miniature, (SMITH-STOCKING, unpublished) appeared which show the same type of sex-linked inheritance. The first evidence suggested that the sex chromosome complex of the female was XX and that of the male was XY. Later however the male soma was found to contain only seven chromosomes, indicating that it has no Y chromosome. The male germ-line possesses two sex chromosomes. One of these is evidently the same as the X in the somatic cells, but the precise nature of the other is obscured by a series of phenomena which are not yet fully analyzed and which need not be reviewed here (METZ 1934). The evidence is consistent, however, with the view that these two undergo the same type of selective segregation as that shown by the autosome pair just considered.

In each of two other species of Sciara a character was found which was inherited in the same way as truncate wings, suggesting that perhaps this unusual chromosome segregation is typical of the genus (METZ 1928, METZ 1929).

The present genetic study was undertaken for the purpose of analyzing the method of segregation of all the chromosomes of one species. It has involved: (1) the securing of new mutant characters, sufficient in number to identify each pair of chromosomes, and (2) analyzing the relationship of the new characters to each other by means of linkage tests to ascertain whether all of the chromosomes observed cytologically (save the "limited" chromosomes) were accounted for genetically. *Sciara coprophila* was used since it is the most satisfactory species for laboratory purposes. Although the sex chromosomes and one pair of autosomes had been previously identified genetically, there remained two pairs of autosomes to be studied.

Because of the difficulty of securing mutant characters, it has been necessary in the present study to make use of some characters which are inconstant and otherwise unsatisfactory. This has not only increased the task of making genetic tests, but has also necessitated presenting here a more detailed description of the experiments than would otherwise be required. An account of culture methods and breeding technique is included, since it has not been fully treated in earlier papers.

CULTURE METHODS AND BREEDING TECHNIQUE

1. *Culture medium*

Sciara is cultured in glass vials one inch wide and four inches deep. These are sterilized and filled to a depth of approximately one inch with an agar solution made by heating together equal parts of agar-agar and water. To insure a dry surface, a small amount of sterilized ground straw is sprinkled into the vials after the medium has solidified. The vials are plugged with cotton. Half pint milk bottles may be used for maintaining mass cultures.

2. *Life history and food*

The genus Sciara belongs to the group of so-called fungus gnats, some species of which inhabit mushroom beds and often become a serious menace to commercial enterprise. The adult of *S. coprophila* is small, dark, and inconspicuous.

The flies are usually cultivated in pair matings, a single female and one or more males being placed in each vial. Since it has been shown that a given female produces offspring from only one male (Moses and Metz 1928), several males are often placed with one female as a precaution against possible sterility. Copulation usually takes place soon after the flies are placed together. The sperms are stored by the female in the spermathecae and the eggs are fertilized one by one as they leave the vagina. They are deposited on the surface of the agar and hatch into small transparent larvae in about six days, at which time they must be fed.

Many types of food have been tried with varying degrees of success. The most satisfactory one found thus far is a mixture consisting of equal parts of animal-poultry yeast, powdered mushroom, and straw. The latter serves to prevent the formation of an impervious surface layer on the cultures. When small larvae are visible (usually ten days after the parent flies have been placed in the vial), a small quantity of food mixture is sprinkled on the surface. This is soon eaten and the supply must be replenished about every second day until pupation begins. Practice alone will demonstrate what quantity of food is required. In general it is better to feed sparingly rather than abundantly, for if cultures are given too much food, the larvae fail to eat all of it, and the excess remains on top of the culture as a loose mixture, to drop out when one attempts to remove the largely eliminated, the new spirals are well established and continue to effect chromosome shortening until metaphase. The two chromatids of each chromosome are associated in the relic spiral, and, as the relic spiral is uncoiled, the two chromatids are twisted about each other (*relational coiling*, see fig. B). The amount of twisting is reduced as the chromatids contract, and at metaphase few twists remain in the chromosomes of most species. The chromonemata of somatic chromosomes are always coiled. Before the relic coils of the previous division are straightened out, the new spirals are well established in each chromatid. There is a tendency for homologous chromosomes to pair in certain species, but intimate association is inhibited by their coiled structure during the entire mitotic cycle.

The resting stage preceding meiosis shows the same type of chromosome structure. The tempo of the early prophase stage is slower than it is in mitosis, and the relic spirals are well straightened out before the daughter chromatids become differentiated and begin to form new minor coils. At this time the affinity between homologous chromosomes permits intimate pairing. The attraction is effective only when the chromosomes are relatively free from coils and before the chromatids of each homologue become differentiated and begin to coil independently. The chiasmata are formed, and the homologous chromosomes tend to repel each other. During this time the minor coils are developed, and during the later stages the *major coils* (plate 1) are superimposed on the minor coils. The reduction in chromosome length between pachytene and metaphase is about 10:1 and may be even greater in certain species. This contraction may be effected by a linear contraction, or perhaps a "minimum" spiral, and by minor and major coiling. Major coils are not an essential feature of meiosis (O'MARA, unpublished) and apparently do not occur in all genera. The major coils do not prevent free separation of chromatids at the first meiotic division. The interphase is so short that the minor spirals, and in some species even the major spirals, persist in the second meiotic division.

The comparison of mitosis and meiosis seems to show the basis for chromosome pairing at meiosis, but a more detailed analysis is necessary for determining the mechanism involved in crossing over. DARLINGTON (1935) has prepared a most logical theory to explain the mechanism of crossing over. The essential features of this theory are based on the following assumptions. The direction of coiling of minor spirals is the same for homologous arms of somatic chromosomes; the direction of coiling is reversed at the spindle fiber attachment point, so that the two arms of a single chromosome always coil in opposite directions; the direction of coiling does not change between the fiber attachment and the distal end of the chromosome in either somatic or meiotic chromosomes; the minor and major spirals in a given chromosome coil in the same direction; and homologous chromosomes pair while still coiled in relic spirals. After the pairing of coiled homologues at meiosis, the chromosomes elongate and produce a "relational" coiling at pachytene. Each chromosome now divides so that relational coiling exists in the chromatids of each homologue. The relational coiling of chromatids is in the same direction in each homologue, and the relational coiling of chromosomes is in the reverse direction (fig. A). The torsional strain induces chromatid breaks which reunite in such a way that crossing over is effected between two of the four chromatids at any one locus. "The torsion which determines relational coiling is removed by crossing over and consequently must determine it."

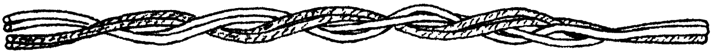


FIGURE A. Relational coiling of homologous chromosomes at pachytene, according to DARLINGTON'S interpretation. The chromatids of each homologue are twisted about each other in the same direction, while the chromosomes twist about each other in the opposite direction.

The theory is logical, but unfortunately most of the underlying assumptions are erroneous. If hypotheses are of more importance than facts (DARLINGTON 1935c), further analysis should be unnecessary; but perhaps a few cytological observations and an analysis of chromosome behavior will be of some value in correlating cytological and genetic behavior.

MINOR COILS AND RELATIONAL COILING IN SOMATIC CHROMOSOMES

The number of coils in somatic chromosomes can be observed only in species with relatively large chromosomes, and even in such species the direction of coiling can not be determined accurately. There does seem to be a tendency for the coiling to be in the same direction from the spindle fiber to the end of the chromosome. The number of minor spirals in a somatic chromosome is about 20–25 in Tradescantia (SAX and SAX 1935) and is about 80 in Fritillaria (DARLINGTON 1935).

Since the direction of coiling in somatic chromosomes can not be determined directly, we must rely upon indirect evidence. This evidence is based on the assumption that the relational coiling of chromatids is determined by the minor coils, either as a result of uncoiling the relic coils (DARLINGTON 1935) or by the formation of new coils in each chromatid at prophase (HUSTED, unpublished).

Relational coiling in the sister chromatids of somatic chromosomes has been studied in Trillium and Vicia. Root tips were fixed in a mixture of absolute alcohol and acetic acid, and after fixing for 15–20 hours, the root tips were smeared in aceto-carmine. In favorable preparations the relational coiling could be observed and the direction of this coiling determined for all chromosomes (figs. B and C).

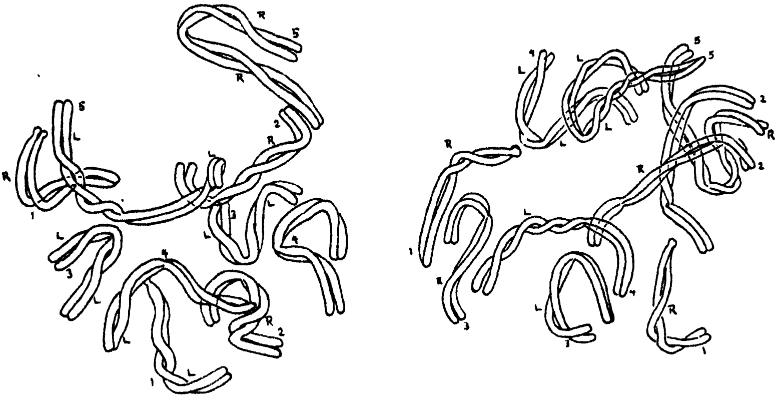


FIGURE B. Somatic chromosomes of *Trillium grandiflorum* showing relational coiling of chromatids. The five pairs of homologous chromosomes are numbered, and the direction of relational coiling is indicated. X800.

Trillium grandiflorum has five pairs of chromosomes: (1) with a nearly terminal fiber attachment, (2) fiber subterminal, (3) sub-median fiber and short arms, (4) sub-median fiber and medium arms and (5) sub-median fiber and long arms (figs. B and C). The corresponding arms of homologous chromosomes could be determined accurately for chromosomes I, II, and V, and with a fair degree of accuracy for chromosomes III and IV. The direction of twisting of the chromatids is easily determined at late prophase or early metaphase.

The direction of relational coiling was obtained for all the chromosomes in each of twenty-five cells. The direction of twisting of chromatids in corresponding arms of homologous chromosomes is shown in table 1.

The total number of chromosome arms with right-handed or clockwise spirals is 113, and with left-handed or counter clockwise twists, 118, while 219 arms showed no twisting of chromatids at the stage of development examined. The homologous chromosomes show an approximately random distribution for direction of relational coiling. Where both of the terminal chromosomes showed relational coiling, the direction of twisting was the same in the two homologues in 9 cells and reversed in 9 cells. The total for all homologous arms was 16 with right-handed twists in both arms, 23 with left-handed twists in both arms, 36 with a right-handed twist in one homologue and a left-handed twist in the other homologue, and 150 pairs of arms which showed relational coiling in only one or in neither arm.

In any single chromosome the direction of relational coiling may be the same in both arms or may be reversed at the spindle fiber attachment point. In the chromosomes which showed relational coiling in both arms,

TABLE 1

Direction of twisting (relational coiling) in sister chromatids for homologous arms of all chromosomes in 25 somatic cells of Trillium. RR=relational coiling right in both homologues; RL or LR =right-handed twists in one homologue and left in the other; LL=left-handed twists in both homologues; O=no twist.

CHROMOSOME		DIRECTION OF TWIST						
		RR	RL OR LR	LL	RO	LO	OO	n
1.	long arm	4	9	5	3	2	2	25
2.	short arm			1	2	1	21	25
	long arm	4	3	2	8	6	2	25
3.	short arm	1		1	1	6	16	25
	long arm	1	5	4	7	2	6	25
4.	short arm		1	2	7	8	7	25
	long arm	3	4	2	5	5	6	25
5.	short arm	2	7	2	9	1	4	25
	long arm	1	7	4	3	5	5	25
		16	36	23	45	36	69	225

the direction was right in both arms of 9 chromosomes, left in both arms of 13 chromosomes, and right in one arm and left in the other arm of 24 chromosomes. In the chromosomes with the same direction of twisting in both arms, the average number of half twists was 1.7 for the long arms and

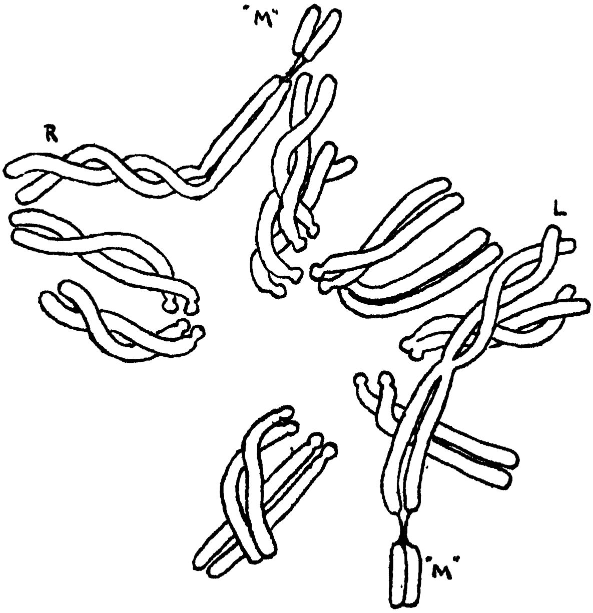


FIGURE C. Somatic chromosomes of *Vicia faba*. The direction of coiling is indicated in the long "M" chromosomes. The short chromosomes are so similar that homologues can not be identified, although the loose pairing may indicate homology. ×2000.

1.3 for the short arms, while in the chromosomes with reversal in direction of twisting in the two arms, the average number of half twists was 1.5 for the long arms and 1.3 for the short arms. These observations indicate that the direction of twisting is approximately at random for either arm of a chromosome and that there is no redistribution of coiling. If coiling is transferred from one arm to the other, there should be considerable reversal of coiling within a single arm, but such reversals are very rare and were observed in only two cases.

There are six pairs of chromosomes in *Vicia faba*, five pairs with nearly terminal fiber attachments and one pair with sub-median fiber attachment points. It is not possible to determine which of the ten short chromosomes are homologues, but the pair of long "M" chromosomes is easily identified, and the two arms can be distinguished easily because the shorter arm has a very clear secondary constriction. We have analyzed the direction of relational coiling in each of the two "M" chromosomes in sixty root tip cells. The results are shown in table 2.

TABLE 2

Direction of relational coiling of chromatids in homologous arms of the "M" chromosomes of Vicia faba at early somatic metaphase.

RR	LR OR RL	LL	RO	LO	OO	n
16	25	13	20	12	34	120

Direction of relational coiling in the two arms of single "M" chromosomes of Vicia

RR	RL OR LR	LL	RO	LO	OO	n
9	18	6	41	33	13	120

The direction of twisting of chromatids about each other seems to be at random for homologous arms, with 29 showing relational coiling in the same direction and 25 showing relational coiling in opposite directions. The 120 "M" chromosomes show relational coiling in 140 arms of which 77 were right and 63 left. In single chromosomes the direction of relational coiling was the same in both arms of 15 chromosomes and reversed in 18. No case was observed where the direction of relational coiling was reversed in the long arm, but in the short arm the direction was reversed on either side of the secondary constriction in several chromosomes. The average number of half twists was found to be the same in the chromosomes with the same direction of relational coiling in both arms and those which had reversed coiling: 1.7 for the long arms and 1.1 for the short arms.

The direction of relational coiling in a single chromosome is not constant during successive cell generations. In root tip cells from a single plant the direction of relational coiling was right in both homologues of six cells, left in both homologues of six cells, and in reverse directions in the homologues of thirteen cells.

THE MAJOR COILS IN THE MEIOTIC CHROMOSOMES OF VICIA

The direction of coiling of major spirals has been determined in a number of species, but no adequate data are available for an analysis of coiling in relation to chiasmata. The meiotic chromosomes of *Vicia faba* have been used because the chiasma frequency is high, and in favorable preparations the direction of the major coils can be determined at all loci in some of the chromosomes. We have made and examined nearly a thousand preparations, but the technical difficulties have made it impossible to get very many data on major coils in this genus.

There are six pairs of chromosomes in *Vicia faba*, one long pair with submedian fiber constrictions, and five shorter pairs with subterminal fiber constrictions. The average chiasma frequency in the material examined was 6.2 for the long "M" chromosomes and 3.0 for each of the five short chromosomes. There is a strong tendency for the chiasmata of the short chromosomes to be localized either near the spindle fiber or near the distal end, and the average number of clearly interstitial chiasmata is only 1.3 for these chromosomes.

The two chromatids of each chromosome are coiled together in a single spiral at meiotic metaphase. There are about 15 major spirals in each of the long chromosomes and about 5 or 6 major spirals in each of the short chromosomes (plate 1). The direction of coiling of a single spiral is seldom reversed between chiasmata, although reversals do occur in the loci containing the fiber attachment in the "M" chromosomes (figs. 2 and 4) and in some internodes of the short chromosomes (figs. 10 and 11). The direction of coiling seems to be at random for corresponding segments of homologous

DESCRIPTION OF PLATE 1

Camera lucida drawings of meiotic chromosomes of *Vicia faba*, showing major coils. From permanent smear preparations. X4000.

Figures 1–4. Bivalents with median fiber attachments Major coiling is at random for homologues segments and for loci on either side of a chiasma. The direction of coiling may reverse at the fiber attachment.

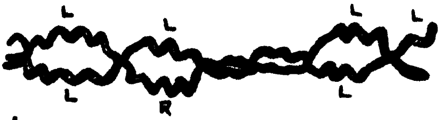
Figures 5–7. Bivalents with sub-terminal fiber attachments.

Figures 8, 9. Anaphase chromosomes.

Figures 10, 11. Two "m" bivalents with reversal of coiling between chiasmata.

Figure 12. Separation of homologues previously associated by two interstitial compensating chiasmata.

Figures 14–17. Anaphase chromosomes showing reversal in coiling in associated chromatids. Opposite directions of coiling in associated chromatids is usually caused by crossingover. The coiling relationship shown in Figure 13 is attributed to a crossover near the terminal spindle fiber rather than equational division.



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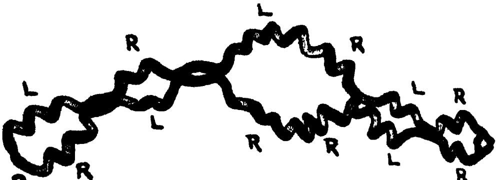

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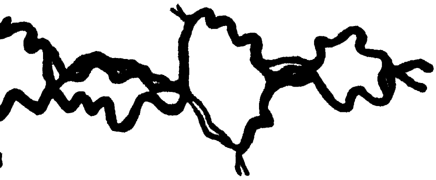

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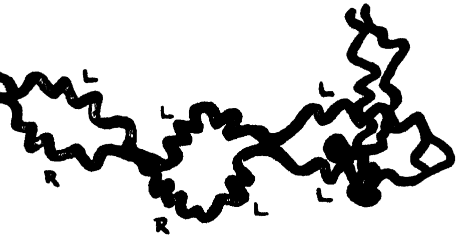

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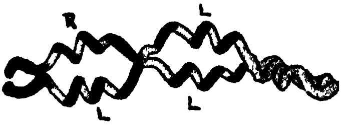

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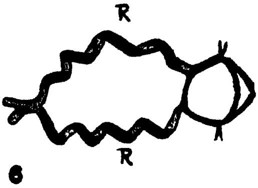

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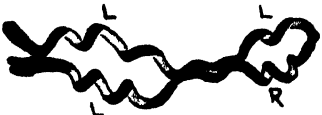

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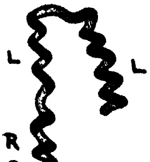

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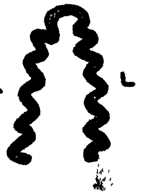

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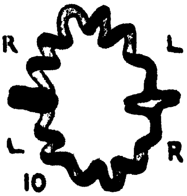

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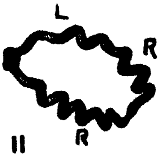

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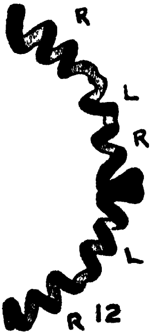

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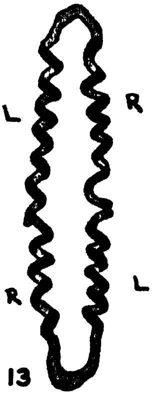

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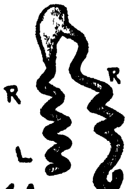

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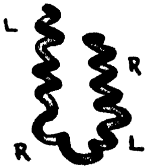

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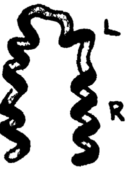

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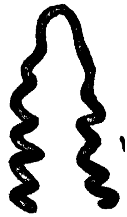

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chromosomes and for loci on either side of a chiasma. The theoretical and actual frequencies are shown in table 3.

TABLE 3

Vicia faba

Direction of coiling on either side of chiasmata, data from both M and m chromosomes.

	THEORETICAL	ACTUAL
LL×LL or RR×RR	2	2
LL×RR or RR×LL	2	1
LR×LR or RL×RL	2	3
LR×RL or RL×LR	2	2
RL×RR or LR×LL etc.	8	8
	16	16

As the chromosomes separate at anaphase, the two chromatids of each chromosome arm separate so that the direction of the major spirals can be determined for each chromatid. We have been able to analyze direction of coiling at anaphase only in the short chromosomes with the nearly terminal fiber attachments (figs. 8 16). The sister chromatids are coiled together in the same direction at metaphase, and if no chiasmata were formed, the two chromatids of an anaphase chromosome should be coiled in the same direction at all loci, with only an occasional double reversal. With random direction of coiling at metaphase, the reversals in coiling of all anaphase chromatids should be twice the chiasma frequency. In 27 anaphase chromosomes the direction of coiling of the two chromatids was the same at all loci in only 5, and the total number of reversals was 32. The reversals in coiling are not so frequent as expected with a chiasma frequency of 3.0. Part of the discrepancy may be attributed to terminal chiasmata. The average number of interstitial chiasmata is 1.3, and the calculated number based on reversal in coiling is 1.2.

Frequently the two chromatids of an anaphase chromosome coil in different directions at all loci (figs. 9, 13, 16). Such configurations are the result of separating a bivalent which has a chiasma very near the spindle fiber and in which the homologues were coiled in opposite directions.

In some cases the two homologues can separate without untangling their chromatids. Figure 12 represents the separation of chromosomes previously associated by two compensating interstitial chiasmata. The association of the very short arms of the "m" chromosomes seems to be effected without chiasma formation (fig. 6). The fiber attachment points are occasionally stretched out from the chromonema, and in these cases they appear to be double (figs. 3 and 6).

One case was found where interlocking of non-homologous chromosomes provides evidence in support of the JANSSEN theory of chiasma formation (fig. 4). The short chromosome is locked in the "M" bivalent distal to the first chiasma from the fiber attachment.

INTERLOCKING OF CHROMATIDS

Whenever two or more chiasmata are formed on one side of the fiber attachment, there should be some interlocking of chromatids at metaphase and early anaphase. In *Vicia faba* there are usually two chiasmata distal to the fiber in the "m" chromosomes, and about three in each arm of the "M" chromosomes. In the "M" chromosomes chromatid interlocking could occur in either arm, but only in the single arms of the "m" chromosomes. In 30 such arms chromatid interlocking was found in 5 cases, or about 17 percent. Chromatid interlocking is shown in figure D (a, d, and e) while a symmetrical "M" bivalent is shown in c. The proportions of these various configurations are of value in an analysis of the mechanism of crossing over.

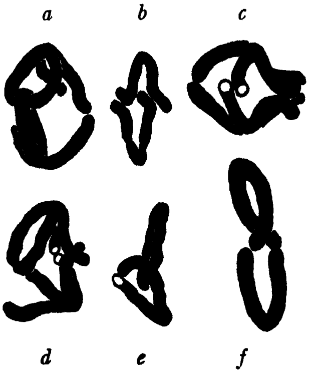

FIGURE D. Bivalent chromosomes of *Vicia faba* at first meiotic anaphase, showing chromatid locking in a, d, and e. A symmetrical (free) bivalent is shown in c.

CHROMOSOME STRUCTURE AND BEHAVIOR IN RELATION TO CROSSING OVER

The analysis of relational coiling of chromatids in somatic chromosomes of Vicia and Trillium provides indirect evidence that the direction of the minor coils is at random for homologous chromosomes, that the direction of minor coiling in the two arms of a single chromosome is at random, and that the direction of coiling is usually the same in any one arm of a somatic chromosome.

The direction of coiling of the major spirals at meiosis has been analyzed in several genera. The paired chromosome arms may coil in the same direction or in opposite directions, apparently at random, in Tradescantia (NEBEL 1932, SAX and HUMPHREY 1934); Sagittaria (SHINKE 1934);

Rhoeo (SAX 1935); Trillium (MATSUURA 1935); Lilium (IWATA 1935); and in Vicia. The direction of coiling may change at chiasmata and occasionally at other loci (HUSKINS and SMITH 1935, MATSUURA 1935) and appears to change at random at chiasmata in Vicia. The major coils may reverse their direction of coiling at the spindle attachment, but there is a strong tendency for the direction of coiling to be the same in both arms of a meiotic chromosome in Tradescantia and Rhoeo. DARLINGTON (1935) assumes that major coiling is in the same direction in homologous chromosomes of Fritillaria, and that the two arms of a single chromosome must coil in opposite directions; but he presents no data or references to support these assumptions, and in the few figures of meiotic chromosomes showing major coils, he fails to indicate the direction of coiling where it is not in accord with his hypothesis (1935b, fig. 1).

The random coiling in the two arms of a somatic chromosome proves that minor coiling is not caused by the rotation of the spindle fiber attachment point. In fact the normal contraction of X-ray induced fragments with no fiber attachments shows that the spindle fiber has no causal effect on chromosome contraction (HUSKINS and HUNTER 1935, RILEY, O'MARA, HUSTED, unpublished). The relational coiling in homologous chromosomes is at random in mitosis. The direction of major spirals is at random, or nearly so, in homologous chromosomes. The direction of coiling of major spirals is not dependent on the direction of coiling of minor spirals, because the minor spirals usually coil in the same direction in any one chromosome arm, while the major spirals may change their direction at a chiasma in the paired arms of homologous chromosomes.

All these observations are contrary to DARLINGTON'S assumptions, and most of them are not in accord with his recent hypothesis on the mechanism of crossing over. There is additional evidence which seems to invalidate this hypothesis.

Several hypotheses have been presented to explain the mechanism of crossing over demanded by JANSSENS' chiasmatypy theory. The first was suggested by MORGAN in 1919, and is based on the assumption that the two homologues are twisted in one direction. Breaks occur in two of the four chromatids, and these reunite in new associations. A modification of this theory was suggested by WILSON and MORGAN in 1920. The second hypothesis was proposed by BELLING (1933). He assumed that the chromosomes are single at the time of pairing; that they form half twists or overlaps at early pachytene, and when the chromomeres of each chromosome split, the connecting fibers may be formed between chromomeres of homologous chromosomes. The third theory is DARLINGTON'S relational coiling hypothesis, which demands that the relational coiling of chromatids causes relational coiling of chromosomes in a reverse direction, and that the torsional strain induced causes chromatid breaks with reunions in new associations.

There is little direct cytological evidence to support any one of these hypotheses, but we can test the different theories by comparing the theoretical chromatid relationships which must be produced with the configurations actually observed.

The only adequate data are those on the chromatid relationships of meiotic chromosomes of Melanoplus (HEARNE and HUSKINS 1935). In the chromosomes with two chiasmata each, there are four types of chromatid relationships: free, chromosomes locked, chromatids locked, and continuous. These types are illustrated by diagrams in figure E. As the meiotic chromosomes begin to separate at anaphase, it is difficult to distinguish the various types of configurations with the exception of chromatid interlocking. In 59 bivalents of Melanoplus there were 24 (40 percent) free, 7 (12 percent) with chromosomes locked, 10 (17 percent) with chromatids locked, and 18 (30 percent)continuous. In early anaphase chromosomes of Vicia 17 percent showed chromatid locking, and in Lilium (MATHER 1935) there was less than 17 percent of chromatid locking.

Where there are two chiasmata on the same side of the fiber attachment, we should expect to obtain the four types of bivalents shown in figure 5. Configurations more complicated than simple chromatid locking are not found in Melanoplus and are rarely found even in species with higher chiasma frequencies. Any valid theory of crossing over should give the four types of bivalents observed.

Let us first examine the theory based on the suggestions of WILSON and MORGAN. The four chromatids lie parallel and twist together in one direction. The torsion causes a break in two non-sister chromatids and a subsequent reunion in a new association. If the torsion is in the same direction at all loci, the greater stress may lie on either side of the chromatid breaks, so that, in effect, the rotation of broken chromatids will be at random. The essential features of torsional strain, the chromatid breaks, types of crossover chromatids and chromatid relationships in the bivalents are shown in figure E and table 4. The application of this hypothesis produces 12.5 percent of free bivalents, 12.5 percent of chromosome locking, 25 percent of chromatid locking, and 50 percent of continuous association of chromatids.

We have attempted to analyze BELLING'S and DARLINGTON'S hypotheses in the same way. In the case of BELLING'S theory, we have assumed that the homologous chromosomes can be twisted about each other at random, or in only one direction. In applying DARLINGTON'S theory we have assumed that the amount of relational coiling in chromatids is the same as the relational coiling of chromosomes, but in the reverse direction (fig.

TABLE 4

Crossovers and types of bivalents resulting from two successive crossovers. Torsion of four parallel chromatids. See diagram in figure E.

TORSION	CHROMATIC BREAKS		CROSSOVER CHROMATIDS	TYPE OF BIVALENT
1–2	A	A	2 non-crossover	Chromatid lock
	B	B	2 compensating	
1–3	A	A	2 non-crossover	Free
	B	B	2 compensating	
1–2	A	A′		
	B	B′	4 non-compensating	Chromatid lock
1–3	A	A′		
	B	B′	4 non-compensating	Chromosome lock
1–2	A	A		
	B	B′	4 compensating	Continuous
1–2	A	A′		
	B	B	8 non-compensating	Continuous
1–3	A	A		
	B	B′	4 non-crossover	Continuous
1–3	A	A′		
	B	B		Continuous

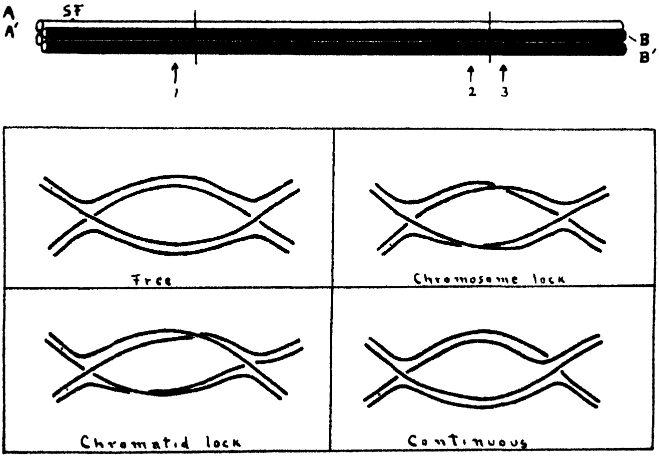

FIGURE E. Pachytene association of chromatids before crossing over, with torsional strains and points of crossing-over indicated. Below are the four types of bivalents produced by the torsion, and the only types observed cytologically in Melanoplus.

A). The configurations produced in these various theories are shown in table 5.

The rotation-torsion hypothesis gives all four types of bivalents observed cytologically, and only these types. If the torsional stress is not at random, the proportions of free, chromosome locked, and chromatid locked bivalents will be changed. But even with the maximum production of free bivalents, the frequency would not equal the percentage observed in

TABLE 5

The configurations produced by different theories of crossing over in chromosomes with two chiasmata distal to the fiber.

HYPOTHESIS	TYPES OF BIVALENTS IN PERCENT				
	FREE	CHROMOSOME LOCK	CHROMATID LOCK	CONTINUOUS	COMPLEX LOCK
WILSON-MORGAN	12½	12½	25	50	0
BELLING random	25	18¾	6¼	25	25
BELLING same	0	0	50	50	0
DARLINGTON	0	0	25	50	25
OBSERVED					
Melanoplus	40	12	17	30	0
Lilium			17		
Vicia			<17		

Melanoplus. However, the theoretical and observed frequencies of the other classes do not deviate so greatly, considering the number of observations and the difficulties in interpreting the chromatid relationships. The percentage of chromatid interlocking in Melanoplus, Lilium, and Vicia is considerably less than the theoretical expectation, but observations based on anaphase figures would fail to detect all the chromatid locking.

This torsion hypothesis will produce no chromosome locking and does produce complex interlocking if twists occur in sister-chromatids or if sister strand crossing over occurs between the two chiasmata.

If BELLING's and DARLINGTON's theories have been interpreted correctly, it appears that neither one is valid. If the homologous chromosomes are twisted at random at early pachytene, BELLING's theory would be expected to produce very few chromatid locks, and 25 percent of complex interlocking. If relational coiling is in the same direction at all loci of the paired chromosome arms, as seems probable, we should expect only chromatid locking and continuous associations of chromatids. BELLING's theory is also difficult to reconcile with the many observations that the chromosomes split at least one cell generation in advance of division.

The application of DARLINGTON's theory gives neither of the first two classes of bivalents and does produce complex interlocking. The cytological evidence also shows that most of DARLINGTON's "observations" regarding relational coiling are incorrect.

The torsion theory based on the suggestions of WILSON and MORGAN seems to be most nearly in accord with the cytological observations. This theory demands the following cytological interpretation. The chromosomes at meiotic prophase elongate until the relic coils of the preceding mitotic division are practically eliminated. There is little or no relational coiling of the chromatids in each chromosome. The homologous chromosomes pair so that all four chromatids are approximately parallel, or at least tend to lie in the same relative quadrant at all loci. The formation of minor or subminor coils is now initiated, and all four chromatids coil in the same direction. The torsion induced by the initiation of the new coiling causes a relational coiling of all four chromatids. This relational coiling produces crossing over at late pachytene or at early diplotene. Much of the relational coiling of the homologues may persist during diplotene, but further contraction of the chromosomes, effected by major and minor coils, eliminates relational coiling at the later stages. The uncoiling of the homologous chromosomes will lead to no complex associations so long as there is no independent relational coiling of the chromatids of each chromosome.

This cytological interpretation of crossing over does not appear to be irreconcilable with the cytological evidence. It is difficult, however, to imagine the mechanism which would produce the precise crossing over demanded by the genetic evidence, and limit it to homologous chromatids, but this difficulty is inherent in any torsion theory of crossing over.

SUMMARY

During somatic prophase the two chromatids of each chromosome are twisted about each other. This relational coiling of chromatids has been analyzed in the somatic chromosomes of *Trillium grandiflorum* and *Vicia faba*. The direction of relational coiling is approximately at random for corresponding arms of homologous chromosomes and for the two arms of a single chromosome. In any one chromosome arm the direction of relational coiling is rarely reversed. There is no evidence that relational coiling is transferred from one arm to the other arm of the same chromosome. It is believed that relational coiling is conditioned by the nature of the minor coils in the somatic chromosomes, so that a study of relational coiling permits an indirect analysis of minor coiling.

At meiosis major spirals are superimposed on the minor coils, at least in a number of plant species. The direction of coiling of these major spirals can be observed directly. In the various species which have been studied, the direction of coiling is approximately at random for homologous chromosomes. In *Vicia faba* the direction of coiling of major spirals is at random on either side of a chiasma. Occasionally it may be reversed at other loci. The direction of coiling of major spirals is not necessarily dependent upon the direction of coiling of the minor spirals.

The various theories of crossing over, based on JANSSENS' partial chiasmatypy hypothesis, have been analyzed in relation to chromosome behavior and chromatid relationships. The torsion theory proposed by

Wilson and Morgan in 1920 seems to be most nearly in accord with the cytological observations.

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HARELIP IN THE HOUSE MOUSE

I. EFFECTS OF THE EXTERNAL AND INTERNAL ENVIRONMENTS

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INTRODUCTION

THE character "harelip and cleft palate" is definitely inherited (REED and SNELL, 1931). Though there are various degrees of expression of harelip and cleft palate, the general condition, regardless of the types of clefts, will henceforth be designated simply as "harelip." Harelip is not inherited as a simple dominant or a simple recessive but seems to depend upon either one recessive gene with modifiers or the cooperation of a small number of cumulative genes. If the first hypothesis is correct, it must be assumed that when the recessive gene for harelip is homozygous, the mouse may show any grade of harelip from the most extensive bilateral clefts to a normal mouth without clefts, depending upon whatever genetic and environmental modifiers had an effect upon the embryo. The phenotypically normal, but genetically harelip, animals will be designated "normal overlaps."

It is usually easy to determine at birth whether a mouse is phenotypically harelip. Occasionally the cleft or clefts are so slight that they are hardly noticeable. Probably in several cases there have been slight lip and palate defects in genetically harelip animals which were not noticed. Such animals would have been erroneously classified as "normal."

Environmental influences greater than ordinary may be suspected in connection with variable characters such as harelip. These effects may be detected if found to occur in correlation with differences in litter size, weight, age of mother, and the like.

EXPRESSION OF HARELIP AFFECTED BY THE EXTERNAL ENVIRONMENT

One can not hope to measure these except with inbred stocks. Following the discovery of harelip in 1930, the stocks have been intensively inbred. At present there are three highly inbred lines and their substrains, all descended from the original harelip albino stock without outcrossing except in the early generations. For the last 10–14 generations a few matings have been made between daughter and father but nearly all have been between sister and brother.

The extent to which these three lines have been inbred and the variation in the percentages of harelip produced as inbreeding proceeded are shown in figure 1. Only the main branches of any of the lines are shown; the less important branches are usually of short duration before becoming extinct for one reason or another. The percentages of harelip in line 1 have increased as inbreeding continued whereas in line 3 they have decreased (down to 0 percent). Each point of each line in figure 1 was determined by the average percentage of harelip in 2–10 litters of the generation plotted. Line 1 has at this time reached a coefficient of inbreeding (WRIGHT's) of over 95 percent; this is equivalent to some fourteen generations of brother

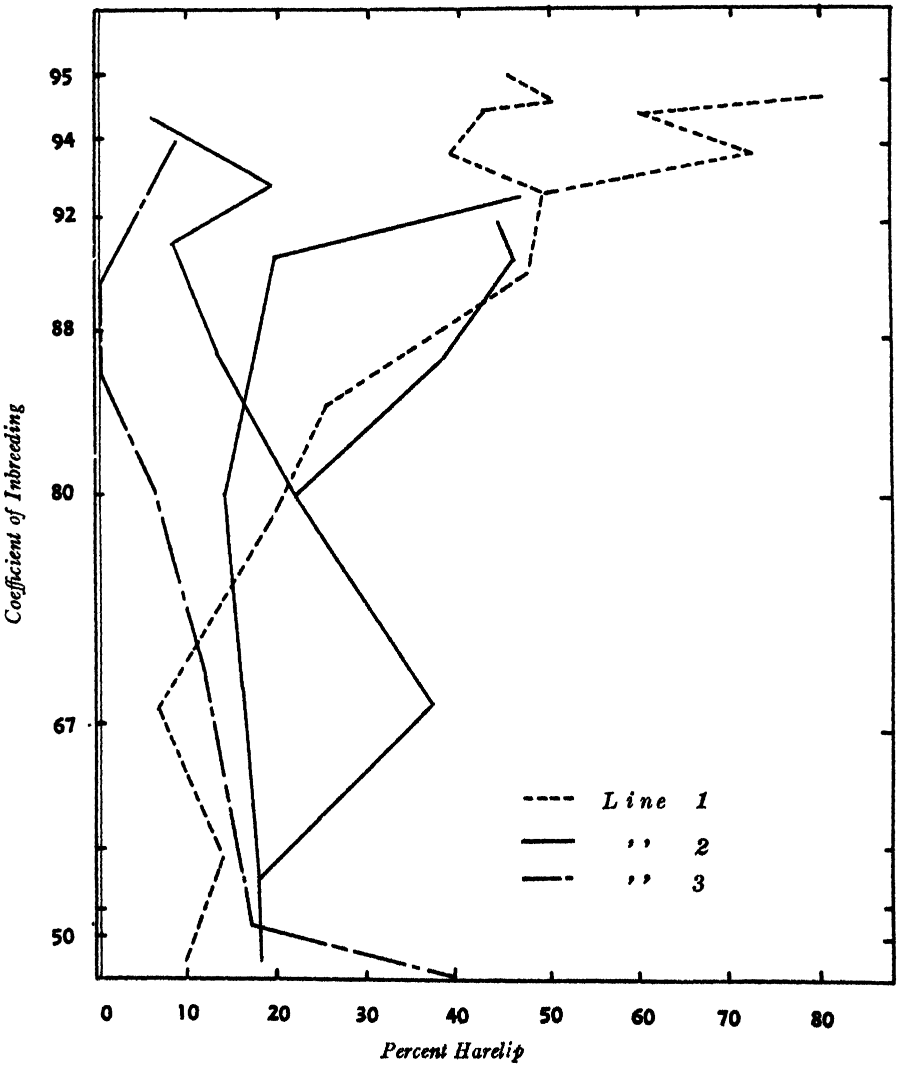

FIGURE 1. The main branches of three highly inbred harelip lines studied in this paper.

by sister inbreeding. We may assume that there is now practically complete homozygosity in each of these lines.

The difference in percentage of harelip between lines 1 and 3, in the last generations at least, is sufficiently great and consistent to indicate real genetic differentiation. In the following paper an attempt will be made to determine whether this difference is due to different combinations of the genes modifying one recessive essential for any harelip production or whether harelip depends upon the cumulative action of several genes of similar value, different groups of which produce characteristic frequencies of harelip.

Our first problem, however, is to test whether some of the variability within any one strain is due to environmental factors. The data for the study of environmental factors are derived from lines 1, 2, and 3 and their sublines (1a, 2a, 2b, etc.). In investigations such as that of the relation between sex and harelip expression, crossbred animals have been included with the inbred animals of lines 1, 2 and 3 but all cases where animals are included that have not been inbred for several generations are specified.

Litter size

It was shown many years ago that litter size is probably the most important single factor in determining the birth weight of guinea pigs, and that the larger the litter, the shorter the gestation period (MINOT 1891, WRIGHT 1921 and 1922, and EATON 1932). Probably the situation is similar in mice; GATES (1925) reports that in mice the birth weights tend to vary inversely with the litter size, while KING (1915) has shown that weight of rats at birth is in direct proportion to the length of the gestation period. Litter size accordingly would seem to be a factor worth investigating in its relation to harelip.

If there is a competition between embryos for some substance particularly concerned with normal development of the jaw and palate, supplied by the mother only in a certain quantity, the smaller the litter the more of the substance for each embryo. Accordingly, small litters should have a lower percentage of harelip than large ones.

Litter size was investigated in 525 litters. The first and second litters of each of the mothers are included and as many following litters in consecutive order as it was possible to include. Each of the litters was observed within 24 hours of birth. All the litters from any one mother had the same father. All the parents were from the inbred stocks (lines 1, 2 and 3) derived in 1930 from Bagg albinos.

Many precautions have been taken in selecting proper litters for the study of environmental effects, with the intention that all material might be strictly comparable. In the statistical treatment, the standard error has been used in preference to the probable error.

TABLE I

Distribution of harelip litters by size and by number of harelip (hp=harelip animals)

GROUP	SIZE OF LITTER	OBSERVED NO. OF LITTERS WITH						TOTAL LITTERS	EXPECTED NO. OF LITTERS WITH						
		0hp	1hp	2hp	3hp	4hp	5hp		0hp	1hp	2hp	3hp	4hp	5hp	6hp
	1	3						3	2.7	0.3					
	2	10	3					13	10.4	2.5	0.1				
	3	24	8	1				33	21.1	10.1	1.7	0.1			
	4	32	6	1				39	24.9	11.8	2.1	0.2			
A	5	25	21	4				50	28.6	16.9	4.0	0.5			
q=.1059	6	33	19	4	4			60	30.7	21.8	6.4	1.0	0.1		
	7	27	20	9	2			58	26.5	22.0	7.8	1.5	0.2		
	8	15	10	6	5			36	14.7	13.8	5.8	1.5	0.2		
	9	10	4	3				17	6.2	6.7	3.2	0.9			
	10		2	7				9	3.2	3.5	1.9	0.6			
	11		1					1	.3	.4	0.3	0.0			
	12	1	2					3	.8	1.1	0.8	0.3			
Total		180	96	35	11			322	169.9	110.9	34.1	6.6	.5		
	1	2	1					3	2.1	0.9					
	2	5	3	1				9	4.6	3.7	0.7				
	3	4	2	3	1			10	3.6	4.6	1.8				
	4	8	4	3	1			16	4.1	6.7	4.1	1.1			
B	5	8	5	9	4	2		28	5.1	10.4	8.4	3.4	0.7		
q=.2889	6	6	6	6	6		1	25	3.2	7.9	8.0	4.4	1.3	0.2	
	7	4	4	13	5	3		29	2.7	7.6	9.2	6.3	2.5	0.6	0.1
	8	1	2	4	3	2	3	15	1.0	3.2	4.5	3.7	1.9	0.6	0.1
	9			2		1		3	0.2	0.5	0.9	0.8	0.5	0.1	0.0
	10		1	2	1	2		6	0.2	0.9	1.5	1.6	1.1	0.6	0.1
Total		38	28	43	21	10	4	144	26.8	46.4	39.1	21.3	8.0	2.1	0.3

TABLE 1 (*Continued*)

GROUP	SIZE OF LITTER	OBSERVED NO. OF LITTERS										TOTAL LITTERS
		0hp	1hp	2hp	3hp	4hp	5hp	6hp	7hp	8hp	9hp	
C q = .4859	1	1	1									2
	2	1	1									2
	3	3	1	3								7
	4	3	2	3	2							10
	5		1	1	5	2						9
	6		1	3	6	2						12
	7				6	1	1	1				9
	8					3		1				4
	9						1	1		1		3
	10											
	11										1	1
Total		8	7	10	19	8	2	3		1	1	59
		EXPECTED NO. OF LITTERS										
C q = .4859	1	1.0	1.0									2.0
	2	0.5	1.0	0.5								2.0
	3	0.9	2.7	2.6	0.8							7.0
	4	0.7	2.6	3.7	2.4	0.6						10.0
	5	0.4	1.5	2.9	2.7	1.3	0.2					9.0
	6	0.2	1.3	3.0	3.8	2.7	1.0					12.0
	7	0.1	0.6	1.6	2.5	2.4	1.4	0.4				9.0
	8	0.0	0.2	0.5	0.9	1.1	0.8	0.4	0.1			4.0
	9		0.1	0.3	0.5	0.8	0.7	0.4	0.2	0.0		3.0
	10											
	11		0.0	0.0	0.1	0.2	0.2	0.2	0.2	0.1	0.0	1.0
Total		3.8	11.0	15.1	13.7	9.1	4.3	1.4	0.5	0.1	0.0	59.0
GRAND TOTALS A–C												
Observed		226	131	88	51	18	6	3		1	1	525
Expected		200.5	168.3	88.3	41.6	17.6	6.4	1.7	0.5	0.1	0.0	525.0

In the quantitative determinations of the amounts of variance affected by various influences such as litter size a smaller population has been used. This is composed of animals from line 1 and its recent branch 1a. This population of 1284 animals will be referred to as the "small population," and will be used for all biserial eta and tetrachoric correlations.

Most environmental factors would act on litter mates alike. Malnutrition, disease and age of mother, if effective, would tend to make litters have excesses of either harelip or normal as the conditions might determine. A method of ascertaining whether or not there are disturbances in the distribution of normal and abnormal animals among the litters has been developed by WRIGHT (1934a) and will be used here. It is possible to calculate the expected occurrence of harelip in litter mates as well as the number of harelip animals expected for each size of litter.

Each mother may be placed in one of three groups in respect to the percentage of harelip young produced in all her litters. In table 1, Group A includes all the litters from mothers which produced up to 21 percent of harelip young (as an average of the fraternity). Group B includes all the litters from mothers which produced from 21 to 40 percent of harelip young. Group C includes all the litters from mothers producing from 41 to 60 percent of harelip young.

The distribution of harelip in litters of each size, expected under random sampling, can be calculated as follows. Let q be the chance of abnormal development in the group in question, and $(1-q)$ the chance of normal development. In litters of 2 the chance that both will be normal is $(1-q)^2$; that one will be harelip and one normal is $2q(1-q)$; and finally that both will be harelip is q^2. In litters of 3 the chances of 0, 1, 2, 3, harelip are the appropriate terms in the expansion $[(1-q)a+qA]^3$ where a stands for normal and A for abnormal. The expectations for larger litters are given by expansion of the appropriate power of the binomial.

The degree of agreement of the grand totals of table 1 is shown in table 2. Note that there were many more litters observed than expected in which there were no harelip offspring and a considerable deficiency of litters containing one harelip animal.

TABLE 2

Relation of the observed number of litters containing 0–9 harelip offspring to the number expected. (hp = harelip)

	0 hp	1 hp	2 hp	3 hp	4 hp	5–9 hp	Total
Observed $(m+x)$	226	131	88	51	18	11	525
Expected (m)	200.5	168.3	88.3	41.6	17.6	8.7	525.0
x^2/m	3.24	8.27	0.00	2.12	0.01	0.61	14.25

There are six classes in the table and two degrees of freedom are lost—one by accepting the total number and one (approximately) by accepting the proportion of harelip in each group. With four degrees of freedom the chance that such deviations would occur from random sampling is less than 0.01 and because of this small probability, the deviations are undoubtedly significant. The deviations are of the order expected if there were a tendency for members of a litter to be similarly affected by environmental factors. Among other factors, age of mother, if significant, could have caused deviations of this order.

Table 3a presents the data for the large population (lines 1, 2 and 3). It will be noted that small litters are deficient in harelip, litters of 5–7 agree with expectation while there is an excess of harelip in large litters. The χ^2 test shows that the correlation between large litters and higher percentage of harelip is undoubtedly significant; total $\chi^2 = 16.04$ with only two degrees of freedom.

TABLE 3a

Significance of the relation between size of litter and number of harelip in the litter (whole population)

Litter size	1–4	5–7	8–12	Total
Observed hp (19.6%) (m+x)	74	345	178	
Expected hp m	103.3	339.3	154.4	
Deviation x	−29.3	+5.7	+23.6	
x^2/m	8.30	0.96	3.62	12.88

$$\chi^2 = \left(1 + \frac{.196}{.804}\right) \Sigma \frac{x^2}{m}$$

$\chi^2 = 1.244 \times 12.88$ Total $\chi^2 = 16.04$

TABLE 3b

Significance of the relation between size of litter and number of harelip in the litter (small population)

Litter size	1–4	5–7	8–12	Total
Observed hp (25.1%) (m+x)	48	179	100	327
Expected hp m	55.3	184.6	87.1	327.0
Deviation x	−7.3	−5.6	+12.9	
x^2/m	0.96	0.17	1.91	3.04

$$\chi^2 = \left(1 + \frac{.251}{.749}\right) \Sigma \frac{x^2}{m}$$

$\chi^2 = 1.335 \times 3.04$. Total $\chi^2 = 4.06$

In the small population the correlation is of the same type as that for the large population although χ^2 is not significant. There can be no doubt, however, that the effect of size of litter is present in the small population even though not in such a pronounced degree (table 3b).

The actual correlation was calculated for the small population (1284 animals). Biserial eta squared is equal to 0.035. This means that 3.5 percent of the total variation in harelip production of the small population is due to the litter size. WRIGHT's formula for biserial eta squared is, eta squared $= \sigma^2/1 + \sigma^2$. The sigma is that for the total group of arrays.

Age of the mother

KING (1917) and others have found a relationship in rats between age of mother and birth weight of young. In all cases the young from mature dams were heavier than those from young females. If the age of the mother is an important factor in the expression of harelip, young mothers might be expected to produce a higher percentage of harelip young than mature ones.

When the mean percentage of harelip produced by mothers of the various ages is calculated, we find larger fluctuations than we should expect from chance alone. The percentage of harelip young is high in the litters of 2 and 4 months old mothers but, by exception, is very low in the case of mothers 3 months old. Subjected to all manner of tests, this low percentage from 3 months old mothers remains. It is always consistent and significant. The percentage for mothers 4–7 months of age is about that found for 2 months old mothers, but for mothers 8–10 months old, the percentage is very low (table 4).

It was thought necessary to study those mothers which were still producing litters at 7–10 months of age. By selecting only those mothers which produced offspring from the time they were about 2 months of age until they were about 7–10 months old we avoid fluctuations in the data resulting from differences in harelip frequency of different fraternities of young. As an illustration, if one mother produced an average of 50 percent of harelip progeny in all her litters but produced none after she was 6 months old, while a second mother averaged only 25 percent harelip, but produced them through 10 months of age, there would be a spurious drop in harelip production after 6 months of age observed in the combined data of the two females. Mothers which are more nearly homozygous for genes influencing harelip might produce higher percentages of harelip but fail to produce young after 6 months of age. Therefore the following calculations are from mothers which produced about one litter a month from the age of 2 months to 10 months.

The lower part of table 4 shows the significant drop in harelip production at 3 months of age. This drop, which is the only statistically significant fluctuation, is impossible to explain physiologically at present. The drop in harelip percentage comes suddenly with mothers 10 weeks of age, while the abrupt rise begins at about 15 weeks. Examination of several groups of females separately shows, consistently, the drop in percentage of harelip produced by 3 months old mothers.

A χ^2 table was made to test whether there is an association between harelip expression and age of mother. In this table there were eight age groups against the two alternatives harelip and not-harelip, seven degrees of freedom, and a total χ^2 of 18.01. It is probable that such a large χ^2 would result from chance factors alone only about once in one hundred times. Therefore there is a significant relationship between the expression of harelip and the age of the mother.

TABLE 4

The percentages of harelip young born to mothers from 2–11 months old

AGE OF MOTHER (MONTHS)	NO. OF LITTERS	HARELIP YOUNG (PERCENT)
2	45	30.9±3.5
3	83	19.6±2.2
4	85	25.2±2.4
5	55	30.9±3.4
6	38	23.5±3.5
7	23	22.0±4.1
8	17	17.2±4.3
9	10	16.1±4.7
10	3	11.3
11	2	0.0

Data from mothers still producing at 7–10 months

AGE OF MOTHER (MONTHS)	NO. LITTERS	HARELIP YOUNG (PERCENT)	DIFFERENCE
2	52	29.9±3.2	
			2.2$^{\sigma}$
3	100	21.2±2.2	
			2.6$^{\sigma}$
4-5	172	28.8±2.0	
			0.7$^{\sigma}$
6-7	95	31.5±3.1	
			1.8$^{\sigma}$
8-10	56	23.3±3.5	

As a descriptive statistic it would be interesting to know how great the correlation is between age of mother and the expression of harelip. The use of the coefficient of correlation (r) is valid only when the regression is rectilinear; therefore it is better to use the correlation ratio (eta), which is suitable for both rectilinear and curvilinear distributions. Biserial eta was used in this case where the two alternatives, harelip and not-harelip, are correlated with the different ages of mother. Biserial eta was found to be 0.164. The error of this correlation is not included, the χ^2 test having shown the relation between expression of harelip and age of mother to be significant. The symbolism and formulae employed in determining eta were taken directly from WRIGHT (1934b, p. 513–514).

We have found eta = 0.164. Eta squared is 0.027, indicating about 3 percent determination of the total variance of harelip by the age of mother. Considering the inexplicable drop in harelip production of 3 months old mothers, this finding of 3 percent variance has not been considered in our final analysis; it is merely indicated that age of mother is a factor, but its quantitative determination is left in abeyance.

WRIGHT's (1934b) valuable paper on polydactyly shows "that immaturity of the mother has a much greater influence on the development of an atavistic little toe by the young than on a number of characters (such as mortality at birth) in which an effect would seem more likely on *a priori* grounds." I assume that the age effect in the case of polydactyly may parallel the age effect on harelip. I assume that if there is an effect of age, it is a direct physiological one acting upon the early embryo.

Time interval between litters

The gestation and nursing periods in mice each take about three weeks. Females often carry a litter while nursing the litter recently born and may, for a period of several months, both carry and nurse successive litters at the same time, weaning one litter at the birth of the next one. Such a reproductive load could conceivably affect expression of harelip. We may investigate this possibility by use of the time interval between successive litters. If a litter is born 3 or 4 weeks after the birth of the previous litter, it is likely that this litter was carried while the previous litter was nursing. If, however, the litter is born 6 weeks after the previous one, this previous litter will have been weaned before gestation of the later one. We may compare the percentages of harelip contained in litters born 3, 4, 5, or 6 or more, weeks after the birth of the previous litter. Percentages of harelip for each litter were not calculated, but the mean percentage of harelip of all litters born at the specified period. The material is all from the small population.

NO. OF LITTERS	NO. OF WEEKS ELAPSED SINCE BIRTH OF THE PREVIOUS LITTER	PERCENT HARELIP
89	3	35.0
25	4	39.8
12	5	44.5
35	6 or more	30.6

We may well combine the data for 3 and 4 weeks (114 litters with 36.0 percent harelip) and for 5 and 6 weeks (47 litters with 34.4 percent harelip). The difference between these percentages could easily be due to chance alone. There is, then, no significant effect of the length of the time interval between litters upon the expression of harelip.

A further related attempt was made to analyze effects of the condition of the mother on the expression of harelip in the young. One might expect to find an association between the nursing of the previous litter and a

TABLE 6

Harelip (*H*) *in relation to age of mother* (*A*) *and birth rank* (*B*)

BIRTH RANK	AGE OF MOTHER IN MONTHS																	
	2		3		4		5		6		7		8		9–10		TOTAL	
	NO. YOUNG	PERCENT HARELIP	NO. YOUNG	PERCENT HARELIP	NO. YOUNG	PERCENT HARELIP	NO YOUNG	PERCENT HARELIP	NO YOUNG	PERCENT HARELIP	NO YOUNG	PERCENT HARELIP	NO. YOUNG	PERCENT HARELIP	NO. YOUNG	PERCENT HARELIP	NO. YOUNG	PERCENT HARELIP
1	81	30.9	100	17.0	36	33.3											217	24.9
2			70	21.4	101	28.7	56	21.4									227	24.7
3					66	42.5	115	27.8	64	26.6	4	25.0					249	31.3
4							41	34.2	15	25.2	63	28.6	7	28.6			262	27.5
5									47	29.8	61	16.4	48	18.8	12	33.3	168	22.0
6											16	25.0	64	17.2	22	27.3	102	20.8
7													3	0.0	44	18.2	47	17.0
8															12	16.7	12	16.7
Total	81	30.9	170	18.8	203	34.0	212	27.4	262	26.3	144	22.9	122	18.2	90	22.2	1284	25.6

higher percentage of harelip in the next litter, if this next litter were *in utero* while the previous litter was nursing. This was not the case. If the previous litter was nursed, 27 of the litters which followed had a higher percentage of harelip than that of each corresponding previous litter; the remainder of the litters that followed (38) had a lower percentage of harelip than each of the corresponding previous litters. If the previous litter was removed at birth and not nursed, 63 litters arriving within the next month contained a higher percentage of harelip than the corresponding previous one, and 62 contained a lower percentage.

Lactation has no apparent influence upon harelip expression.

Birth rank

The correlation between expression of harelip and age of mother ($\eta_{HA} = -.16$) allows us to be fairly certain that a correlation between birth rank and expression of harelip will be found because age of mother and birth rank bear an obvious relationship to each other. The correlation between birth rank and expression of harelip proved to be $\eta_{HB} = -.12$. This is not as great as the correlation between age of mother and harelip expression and indicates that age of mother and not birth rank is a factor involved in the variation of harelip expression. Table 6 presents the data concerned with both birth rank and age of mother.

The correlation between age of mother and birth rank is of course high ($r_{AB} = +.96$). It is concluded that birth rank has no effect on the expression of harelip.

Seasonal variation

It would be reasonable to assume that a character such as harelip might be influenced in its expression by seasonal changes. The temperature of the mouse room was held fairly near 70°F during the winter months, but through late spring, summer, and autumn there were considerable fluctuations due to the effect of heat from outside. In spite of such temperature and seasonal changes there seems to have been no significant variation in the percentage of harelip young (table 7).

Though there seem to be no seasonal differences of a regular sequence in harelip production it might be well to investigate periods of a shorter duration. Some care was exercised in studying day-to-day periods but no significant fluctuations were found in the proportions of harelip offspring produced in the shorter periods.

Feed

The only feed used was a balanced ration sold as a fox chow. Fresh water was always present. As no vegetables were given, the feeding may be considered a constant factor which would have an equal effect, if any, on all the matings.

Uterine resorption

It is conceivable that harelip embryos are absorbed *in utero* in cases where the condition might be serious enough to be lethal at early stages. Line 2 has been so derived that all animals of the last few generations are over 90 percent inbred. We find that this line is producing about 15 percent harelip in these generations, and that the average litter size is 6.8 (32 litters). This is a large litter size for mice inbred to such an extent and in which there has been no selection for litter size. It is probable that there could have been but a very small prenatal death rate of harelip zygotes after implantation in this line at least.

TABLE 7

Absence of relationship between the season of the year and the percentage of harelip

SEASON	NO. OF LITTERS	PERCENT HARELIP
December–February	75	23.3±2.6
March–May	142	24.3±2.0
June–August	122	22.0±1.8
September–November	30	30.5±4.1
December–May	217	23.9±1.6
June–November	152	23.6±1.7
March–August	264	23.2±1.4
September–February	105	25.3±2.2

The difference between the incidence of harelip in line 2 (15 percent) and in line 1 (50 percent), with the litter sizes as they are, could be explained only on some assumption other than that of a difference in resorption. A study of the embryology of harelip (REED, 1933) revealed no evidence of differential prenatal resorption of the extreme cases of harelip.

It seems improbable to the writer that there is early zygotic elimination of harelip animals. If there is zygotic elimination it may be discovered in the future if a close linkage of harelip with some "regular" character is found.

We have now investigated, within the limits of our data, the important agencies of the external environment which might influence the expression of the character. We have found no discernible effect of seasonal fluctuations, feed, birth rank, or uterine resorption. There is evidence that the condition of the mother, as measured by age, has some effect and that litter size has an effect of 3.5 percent on the total variance of harelip.

EXPRESSION OF HARELIP MODIFIED BY INTERNAL ENVIRONMENT

There were 548 males to 460 females with some type of cleft, and 272 clefts of the left side alone and 218 of the right side alone in all the harelip populations. The difference in each of the comparisons (sex and symmetry) is statistically significant.

Sex

Taking all the litters in the harelip stock in which all members were sexed at birth (undepleted litters), we find a total of 735 males to 754 females, including both harelip and normal animals. We have noticed a marked excess of males among the harelip animals, but a slight excess of females when all animals are considered; it follows that among the non-harelip there must be an excess of females. There were 412 normal females to 320 normal males. Most of these excess females are probably normal overlaps for harelip.

In human races the fact is unquestionable that harelip occurs with greater frequency in males than in females, and on the left side than on the right. This agrees with the observations on mice. In man the most serious cases are significantly more common among males than among females (SANDERS, loc. cit.) and this is perhaps true in mice. When all grades of clefts are grouped, there are 54.4 percent males; but if only the severe cases are considered there are 55.5 percent males. Further, there is an eye defect associated with the harelip in my stocks which is perhaps another type of expression of the character. It agrees in showing an excess of affected individuals of the male sex. Of the total of 203 animals with eye defects, 68 were sexed; 51 were males and only 17 were females.

The association of harelip and these eye defects is statistically significant. In the litters in which animals with eye defects appeared, there were 60 animals with both eye defects and harelip, 143 with eye defects but no harelip, 150 with no eye defects but with harelip, and 616 animals without either eye defects or harelip ($p_1 - p_2$ is equal to $.100 \pm .032$ where p_1 = percentage of eye defects in harelip animals). The eye defect is similar to that found by other workers in other strains of mice and appeared in the harelip strains. Its relation to defects reported by other workers is not known. One or both eyelids may be open at birth, often followed by considerable damage to the adult eye.

In man the usual interpretation of the sex and symmetry differences is that males are weaker before birth and that the left side develops more slowly than the right (SANDERS, 1934); therefore the excess of male and left side harelip. As we have no reason to assume that more males than females have the genetic basis for harelip, we may suggest that owing to weaker development of the male, the genotype for harelip can express itself there more often than it can in females, where the threshold is not so readily exceeded.

Cyclopia in man and lower mammals appears more frequently in females than males (WRIGHT 1934a). The threshold here is crossed more easily in females than in males whereas with harelip the threshold is more easily exceeded in males. Presumably the two "thresholds" have quite different biological bases.

We may calculate the effect of sex on the total variance of harelip expression. In the small population (1284 animals) in which we determined the amount of variance due to age of mother, there were 166 harelip males, 476 non-harelip males, 135 harelip females and 507 non-harelip females. The harelip males constituted 25.8 percent of all the males and the harelip females 21.0 percent of all females in the small population.

We shall assume that there is a normal distribution of factor complexes underlying the dichotomy of harelip *versus* not-harelip on a scale in which the factors have additive effects. Then if σ is taken as the unit of measurement we may find the value of σ^2 after determining the means on our normal curve for males and for females. The mean of the males, and in like manner for the females, is found to be the value of the inverse probability integral of the percentage of males which are harelip minus one-half. Thus,

percent harelip (q)		$q-\frac{1}{2}$	$\text{prf}^{-1}\,(q-\frac{1}{2})$
♂♂	25.8	24.2	.650 = ♂ mean
♀♀	21.0	29.0	.806 = ♀ mean

Then,

$$\tfrac{1}{4}(m\,♀ - m\,♂)^2 = \sigma_{sex}^2$$

$$\tfrac{1}{4}(.806 - .650)^2 = .061$$

It will be recalled that the correlation *squared* between harelip and size of litter, for instance, is an indicator of the total effect of size of litter on harelip expression in the particular population which we have studied. For sex we found $\sigma^2 = .061$ so η^2 must equal 0.058. There is then a correlation, η^2, of nearly 6 percent between harelip expression and sex.

Asymmetry

The higher frequency of left clefts (55.5 percent) contrasted with right clefts (44.5 percent) brings up the problem of asymmetry.

With harelip a small portion of the asymmetry is inherited as there are significantly more clefts of the left side alone than of the right side alone. CASTLE (1906) found that a majority of his polydactylous guinea pigs were sinistral, but that there was no specificity in transmission, and his endeavor to increase the sinistrality by selection was unsuccessful.

Harelip in mice (and probably in man) behaves in just this way. From animals 90 percent inbred one can predict that, on the average, more left than right clefts will appear in their offspring, but it is found that there is no comprehensible order in the appearance of the left and right clefts. If there were particular genes for normal (or abnormal) development of the left or right side of the face, one would expect that, as inbreeding proceeds, there would be a gradual segregation so that if unilateral clefts continued to appear they would be more often on one side of the face in any inbred line. The most highly inbred lines (table 10) show no tendency for clefts of either the right or left sides alone to become established in any inbred line. The conclusion that the abnormality is inherited but that the asymmetry is not seems to be justified if we allow for the exception of the excess of left clefts.

TABLE 10

Absence of inheritance of left or right asymmetry (WRIGHT'S *coefficient of inbreeding*)

NO. OF ANIMALS IN FRATERNITY	COEFFICIENT OF INBREEDING	LEFT CLEFT	RIGHT CLEFT
LINE 1 (IN PART)			
6	.594	0	0
39	.672	2	0
13	.734	1	0
10	.785	0	1
36	.826	3	0
10	.859	1	0
24	.886	4	1
54	.908	1	0
60	.925	0	2
50	.940	0	2
42	.951	2	1
LINE 2 (IN PART)			
21	.785	2	0
55	.826	4	8
51	.859	3	0
85	.886	3	2
46	.908	3	2
102	.925	11	7
20	.940	0	0

There is another interesting observation on asymmetry. The eye defect associated with harelip showed an excess of affected males as did harelip itself; there is agreement as far as sex is concerned, but the asymmetry is exactly the opposite. There are more eye defects of the right side alone than of the left side, whereas with harelip the majority was on the left. There were 98 animals with the defects of the right eye, 60 with defects of the left eye and 45 with defects of both eyes.

WRIGHT'S (1934a) analysis of otocephaly shows no greater percentage of high grades of otocephaly among the more prevalent otocephalic females than among the fewer otocephalic males. With harelip we have seen that the percentage of higher grades is slightly greater (though not significantly so) among the males than among the females.

There is at least one distinction between the behavior of harelip and otocephaly. As the total frequency of harelip increases, the grade of the abnormality also increases; whereas with otocephaly, the grade of abnormality tends to decrease as the total frequency increases. In the common inbred harelip strains (high harelip frequency) 52.5 percent of the harelip animals were of the two highest grades (most severe cases), but of the various F_2 and double outcross harelip animals (low harelip frequency) only 31.5 percent were of the two highest grades. An arbitrary, but concrete, system of five grades was used.

With inbreeding the percentage of extreme cases of harelip appears to increase even though the absolute percentage of harelip of all grades may be dropping. In three harelip lines (2a, 2b, and 3) the percentage of harelip dropped with inbreeding, but the percentage of harelip animals of the two highest grades probably rises in an absolute sense (table 11).

TABLE 11

Data for lines 2a, 2b, and 3 of comparable generations

COEFFICIENT OF INBREEDING	PERCENT HARELIP	PERCENT OF TOTAL HARELIP OF 2 HIGHEST GRADES
70±	22.3	40.6
80±	15.5	74.3
90±	10 5	58.3

In line 1a, in which the percentage of harelip has been rising, the percentage of cases which were of the two highest grades has also increased (table 12).

TABLE 12

Increase of frequency and severity of harelip in line 1a

COEFFICIENT OF INBREEDING (PERCENT HOMOZYGOSIS)	PERCENT HARELIP	PERCENT OF TOTAL HARELIP OF 2 HIGHEST GRADES	NO. OF HARELIP
.734	7.7	0	1
.785	20.0	50	2
.826	26.3	60	5
.859	45.0	66	3
.886	46.0	55	11
.908	71.5	93	15
.925	59.3	100	16
.940	79.4	96	23

It might be expected that the fraternities of young among which there were the highest percentages of harelip would also show higher grades of harelip than fraternities with low percentages of harelip. This is not necessarily true because the most severe cases usually become predominant over the lower grades of harelip only after considerable inbreeding, whereas high percentages of harelip may or may not accompany extended inbreeding. It was found that the 280 harelip born in fraternities of less than 30 percent harelip were of the lower grades in 167 instances, and of the two highest grades in 113 instances. Of the 301 harelip animals born in fraternities of over 30 percent harelip, 149 were of the lower grades and 152 of the higher grades. From the fourfold table constructed with these data, we find that $\chi^2 = 5.3$; there is one degree of freedom. There is, therefore, probably a significant association between the two highest grades of harelip and the fraternities with over 30 percent harelip.

We have found that there is no tendency for the asymmetry (side affected) to become fixed with increased inbreeding. Late inbred generations still produce left and right clefts in about the same proportion as did earlier generations. With inbreeding the variability of the expression of harelip decreases; that is, with inbreeding there are fewer unilateral clefts and more bilateral (52.5 percent extreme cases in inbred stocks but only 31.5 percent extreme cases in crossbred stocks). Though the variability of harelip expression (severeness) always seems to decrease it is clear from tables 11 and 12 and figure 1 that as the inbreeding goes on the percentage of harelip may either increase or decrease. As inbreeding progresses the character is expressed more completely and severely irrespective of the percentage of harelip.

ALLOCATION OF THE RELATIVE INFLUENCES OF ENVIRONMENTAL AND GENETIC FACTORS

WRIGHT has developed methods for determining the relative influences of heredity and environment on the variation of characters similar to harelip. It is not easy to apply some of the methods to characters which are lethal and overlap as does harelip, but the following attempts have been made. I am deeply grateful to Professor WRIGHT for advice and assistance given while the paper was being written.

The correlation between parent and offspring could be used to determine the relative influence of heredity in regard to variation of the character. Unfortunately the parents do not have visible harelip, so it is impossible to make a direct parent-offspring correlation between individual parents and offspring. It is possible to determine in a qualitative way whether or not there is some correlation between parent and offspring by finding the ordinary correlation coefficient between the percentage of harelip in the fraternity of the parent and the percentage in the fraternity of the offspring.

The correlation between the percentage of harlip in the fraternity (large population) in which the mother was born and the percentage of harelip she gave was $r = +.333 \pm .093$ while that between the percentage of harelip in the fraternity in which the father was born and the percentage he sired was $r = +.186 \pm .104$. The average of these two correlations is $r = +.26 \pm .07$ which is fairly large and certainly significant. There is, then, a correlation between the percentage of harelip in the fraternity of the parent and the percentage in the fraternity of the offspring, though we can not use the above figures as a direct quantitative test of the amount of variance due to heredity.

What portion of the variability in our population of 1284 animals due to both heredity and environment is common to substrains? We know that the differences common to substrains, but not common to the whole small population, would be wholly genetic. It will be shown shortly that the variation common to sibships is equal to 10 percent. This *includes* the variation common to substrains, and this substrain variation should be equal to 10 percent or less.

The amount of variation common to substrains, but not to the whole population, can be calculated. The population of 1284 animals was divided into its substrains (the family tree partitioned into its branches or groups of fifty or more animals), the mean found for each substrain, $\mathrm{prf}^{-1}(q-1/2)$, and finally biserial eta squared for the total groups. Biserial eta squared was found to be 0.109; therefore about 11 percent of the total variability is common to substrains. The 10 percent variation, common to both substrains and sibships, theoretically should include this 11 percent common to substrains. The reason for the discrepancy is that there is apparently little, if any, variability common to sibships. Otherwise the agreement is as close as could be expected.

If variation were common to sibships, it would probably be divided between genetic differences of sibships and persistent conditions of the mother. Table 3 of the following paper contains evidence that there are probably no persistent conditions of the mother which affect harelip (age of mother, etc., are not persistent conditions). We may conclude that about 11 percent of the total variation in harelip expression is common to substrains and that little if any of the total is common to sibships alone.

The determination of the 10 percent of variation common to sibships and substrains was found by comparing consecutive litters of the sibships. It has just been noted that the variation is practically all common to substrains and none to sibships. The present determination of 10 percent is useful as a check on the previous finding of 11 percent for substrains. The comparison between each individual of an earlier litter and each individual in the next litter was carried out. If there were 2 harelip and 3 non-harelip in the earlier litter and 1 harelip and 4 non-harelip in the next litter, we could form a 2×2 table such as table 8. The actual data for our small population (1284 animals) are in table 9. It is necessary to use the tetrachloric correlation for a normal frequency surface (KELLEY 1924). The correlation was found to be $r_t = +.100$ or 10 percent.

TABLE 8 TABLE 9

Correlations between individuals of different successive litters from the same mating

		ILLUSTRATIVE LATER LITTER				ACTUAL DATA LATER LITTER		
		NOT HARELIP	HARELIP	TOTALS		NOT HARELIP	HARELIP	TOTALS
Earlier Litter	Not harelip	12	3	15	Not harelip	3402	1097	4499
	Harelip	8	2	10	Harelip	1121	485	1606
	Totals	20	5	25	Totals	4523	1582	6105
							$r_t = +.100$	

The analysis of variation common to litters but not to sibships is now possible. If we compare individuals within each litter of the small population in a manner somewhat similar to that used in comparing litters of each sibship we find that the amount of the total variation common to litter mates, and including that common to substrains, is 21 percent. We know already that the variation common to substrains is 11 percent so we may subtract this from 21 percent and get 10 percent of the total variation which is common to litters but not to sibships or substrains. The variance common to litters is divided into 4 percent due to the effect of size of litter and 6 percent miscellaneous effects as yet unaccounted for.

The variation peculiar to the individual is a result in small part of its sex (6 percent) and of any segregation within litters. The amount of segregation should be slight as all members of each litter are certainly homozygous for the main harelip genes and probably for the same set of modifiers in each litter. Our residual, the environmental factors affecting the individual, accounts for nearly 75 percent of the total variation in harelip expression of this population which is approximately homozygous. It is interesting that such intangible factors as accidents of implantation should play such an important part in the non-genetic variation of an organism.

We may conclude that the small population studied quantitatively (1,284 animals) was practically homozygous and that the variation in the expression of harelip was due in small part to sex, litter size and age of mother, etc., but in the major portion to intangible chance factors working from within the mother but not correlated with her activities. Such intangible factors might be accidents of implantation, proximity of embryos, blood supply, etc.

Stated in another way, if all members of late generations of an inbred line possess the same genotype for harelip, whether the individual will be phenotypically harelip depends in small measure upon its sex, the litter size, the age of its mother, etc., while the main determining factors are those of accident or chance. We have no knowledge as yet just which chance factors are most effective.

Thus harelip is similar to white spotting in the guinea pig (WRIGHT 1920) in which, after homozygosis has been reached, further variation (which may be quite considerable) is due mainly to accidental influences affecting each individual more or less independently of his sibs. Such is the case with both white spotting and harelip. The similarity in behavior of harelip and of otocephaly in the guinea pig is even more pronounced.

SUMMARY

In mice there are differences in harelip expression resulting from the action of both environmental and genetic factors. Differences in harelip expression depend upon the sex of the individual, the size of the litter in which the individual was born, the age of its mother, asymmetry of the clefts, and in large part upon intangible chance factors. The variation in harelip expression resulting from these intangible chance factors may be best studied in populations in which all members are genetically harelip.

In a population of 1,284 mice from highly inbred families there was approximate homozygosis of genetic factors including those for harelip. However, non-harelip animals still appear and there is considerable variation among the animals which are phenotypically harelip. The allocation of the effects of the various factors controlling expression of harelip in these families is considered to be of about this order:

Variation common to substrains		11 percent
(but not common to the whole small population)		
Wholly genetic		
Variation common to sibships but not to substrains		0 percent
Genetic differences between sibships Persistent conditions of the mother	little, or none	
Variation common to litters but not to sibships		10 percent
Size of litter	4 percent	
Condition of mother at a given time		
Age of mother	?	
Season	0 percent	
Miscellaneous	6 percent	
Variation peculiar to the individual		79 percent
Sex	6 percent	
Individual genetic factors (slight)		
(segregation within litters)		

Factors of the environment (residual)	73 percent	
Possibly { Accidents of implantation Proximity of embryos Blood supply		
		100 percent

It is possible to simplify our presentation of the results in this fashion:

1. Variation due to hereditary differences		
a. Sex	6 percent	17 percent
b. Substrain	11 percent	
2. Variation due to environmental differences		
a. Litter size	4 percent	83 percent
b. Age of mother	?	
c. Miscellaneous tangible factors	6 percent	
d. Intangible factors	73 percent	

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THE NATURE OF THE INTERACTION OF GENES AFFECTING FOUR QUANTITATIVE CHARACTERS IN A CROSS BETWEEN HORDEUM DEFICIENS AND HORDEUM VULGARE

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INTRODUCTION

QUANTITATIVE characters are of extreme importance in economic plants. Because of difficulties involved in studying the inheritance of these characters less is known concerning their genetics than is the case for the qualitative characters.

SAX (1923), GRIFFEE (1925) and SIRKS (1925) were the first to study factors for quantitative characters by means of their association with factors for the production of qualitative characters. This method was used later by IMMER (1927). LINDSTROM (1926, 1928, 1929, 1931), who has studied the linkage between genes for quantitative and those for qualitative characters in the tomato, was the first to point out the possibilities of studying the inheritance of genes differentiating quantitative characters by means of their linkage with genes differentiating qualitative characters. LINDSTROM's studies furnish rather conclusive evidence that major factors for size of fruit occur in the tomato. HAYES and HARLAN (1920) showed that there were at least three factor pairs affecting internode length in the spikes of barley. They found that these genes had different effects and were cumulative. Some long and short internode types may be differentiated by one factor pair and others by two or three. WEXELSEN (1934) studying genes influencing internode length of the barley spike substantiated these results and pointed out that the genes are not necessarily alike in their dominance relationships, since L_1, L_2 and L_3 were found to be intermediate in a heterozygous condition, L_4 to be nearer to the short type, and L_5 nearer to the long type. RASMUSSON (1935) in a study of quantitative characters in Pisum demonstrated that two main factors, both showing partial dominance towards lateness of maturity, were at work in the material investigated. He estimated that they were responsible for about half the genic variation within the F_2 populations, whereas the other half was believed to be due to modifiers. He found that these genes were not strictly additive in their combined effect and this supported his interaction hypothesis.

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This article reports a study of the effect of genes associated with color of glumes, type of spike and habit of growth upon yield of seed per plant, number of spikes per plant, height of plant, and length of awn. These include genes differentiating the qualitative characters in question as well as those which may be linked with them and which affect the quantitative characters being studied. It is apparent then that in part at least the effect of the differential genes in certain chromosomes is being measured, and it should be possible to establish the nature of the interactions of the genes located in different chromosomes.

MATERIALS

The material consists of data from F_1, F_2 and F_3 generations of a cross of B 1 and Brachytic. The contrasted characters of the parents are as follows:

Character	*B 1*	*Brachytic*
Color of Glumes	Black	White
Type of spike	deficiens	vulgare
Type of growth	normal	brachytic
Yield of seed per plant in grams	1.9 ± .07	4.0 ± .25
Number of spikes per plant	3.8 ± .11	4.7 ± .25
Height of plant in inches	25.6 ± .24	16.1 ± .15
Length of awn in mm	141.6 ± 1.19	71.0 ± .71

B 1 is a standard stock used in linkage studies at the University of Minnesota (DAANE 1931) and has been carried from individual plant selections in the crossing plots. The parent material used as checks in this study was from the progeny of plants used in making the cross. The crosses were made by Dr. F. J. STEVENSON. The Brachytic parent was obtained from Dr. L. J. STADLER, University of Missouri, and was selected by him from a population of the variety Himalaya, the seed of which originally came from the Montana Experiment Station.

METHODS

Planting, harvesting and measuring. The F_1 and F_2 were grown in the summer of 1932 and the F_3 was grown in 1934 in 6 foot rows and spaced 3 inches apart within the row. The B 1 and Brachytic parents were grown as a check in rows adjacent to the F_1. The progeny from nine plants of the B 1 parent were grown with the 20 F_2 families, the nine B 1 families being distributed at random throughout the 20 F_2 families. As a further check the progeny from one plant of the Brachytic parent were grown at the end of the series of plantings adjacent to the progeny from one plant of the B 1 parent. Thus since the F_1 was grown some distance from the F_2 plants any comparison of the two will have to be made through the parents. At the time of harvest each plant was pulled, its height measured in inches, and the spikes cut off and placed in an envelope. During the following winter the plants were classified; and the number of spikes per plant, the length of awns in millimeters, and the weight of seed in grams were recorded. In the F_3, 32 seeds of each F_2 plant were sown and later notes on the mature plants were taken in the field. Each family was studied on the individual plant basis and classified as homozygous or segregating for each of the three qualitative characters involved.

Statistical methods. First, the data were separated into 27 different genotypes on the basis of the factor pairs differentiating the three qualitative characters. Then the sums of squares, variances and regression coefficients were obtained for each genotype. The variances used in calculating the t values were corrected on the basis of the regression coefficients, FISHER (1934, p. 257). The variances for weight of seed per plant were corrected on the basis of the regression coefficients of weight of seed on the number of spikes; those for number of spikes on the basis of the regression coefficients of number of spikes on weight of seed; those for height of plant on the basis of the regression coefficients of the height of plant on length of awn; and those for length of awn on the basis of the regression coefficients of length of awn on height of plant.

From the data analysed in this manner it was found that the variances from the homozygous black, the heterozygous black, and the homozygous white glume segregates did not differ materially. Therefore a generalized standard deviation would be applicable. Consequently the total variance for comparing plants of the genotypes *BB*, *Bb*, and *bb* was obtained by adding the sums of squares for each of the nine genotypes resulting from a segregation of the genes differentiating type of spike and habit of growth and dividing by the appropriate degrees of freedom. The method is illustrated in the following tabulation for weight of seed per plant:

GENOTYPE	N	D.F.	NUMBER OF SPIKES (x) $S(X^2)-N\bar{X}^2$	$S(XY)-N\bar{X}\bar{Y}$	WEIGHT OF SEED (y) $S(Y^2)-N\bar{Y}^2$	REGRESSION COEFFICIENTS Y on X	D.F.	REDUCED $S(Y^2)-N\bar{Y}^2$
vvBrBr	61	60	489.443	503.000	997.500	1.028	59	480.567
vvBrbr	130	129	846.531	1183.417	2488.324	1.398	128	833.954
vvbrbr	64	63	181.484	149.142	329.151	.822	62	206.587
VvBrBr	130	129	1578.992	1151.002	999.069	.729	128	160.050
VvBrbr	249	248	2375.406	1847.454	1864.521	.778	247	427.678
Vvbrbr	130	129	787.700	441.540	333.907	.561	128	86.405
VVBrBr	78	77	805.962	510.542	412.707	.633	76	89.300
VVBrbr	109	108	884.991	606.767	501.801	.686	107	85.789
VVbrbr	54	53	257.500	131.617	82.703	.511	52	15.430
Total							987	2385.760

The data are shown to 3 places to the right of the decimal point but in obtaining the constants used in this study the figures were carried to six places to the right of the decimal point. As can be seen from the tabulation this method of obtaining the variances took out the differences between genotypes and consequently the variance obtained was that within genotypes. For making comparisons between the nine genotypes resulting from segregation of the genes differentiating type of spike and habit of growth the variance was obtained for each by adjusting the sum of squares on the basis of their respective regression coefficients. The reduced sums of squares are shown in the last column of the above tabulation and of course the variance for each genotype is found by dividing the reduced sums of squares by the degrees of freedom left after subtracting from the original degrees of freedom the one accounted for by the regression coefficient. The t values to determine the significance of means and the t values to determine the significance of regression coefficients were obtained by use of methods given by FISHER, (1934) pp. 120 and 138 respectively. In determining whether differences between variances were statistically significant Fisher's formula (1934, p. 214) was employed. Differences giving odds as great as or greater than 19:1 against the deviations noted as being due to the errors of random sampling were considered as statistically significant.

That the regressions were obtained for each of the nine genotypes separately and adjustment of the variance for each genotype was made on the basis of its respective regression coefficient is important in that it shows what components of the variability are being controlled. By such a method the variability that is partially controlled is that due to environment and the genes independently inherited from those linked with and differentiating type of spike and habit of growth. For illustration let us consider the variances for weight of seeds per plant in which it was found that the general correlation coefficient between weight of seed and number of spikes within phenotypes was .81. This shows, as would be expected that the number of spikes per plant affects materially the yield of the plant. Now, since the purpose of this study is to measure differences between genotypes it would be desirable to have the number of spikes held constant within genotypes in so far as it affects yield. This would in no way interfere with the differences in number of spikes between genotypes, but would aid in the study of the genes differentiating the qualitative characters involved and any genes that might be linked with them. The completeness with which the effect of the linked genes is measured depends of course upon the closeness of the linkage relationship.

EXPERIMENTAL DATA

Inheritance of Color of Glumes, Type of Spikes and Habit of Growth, and their Linkage Relationships

A knowledge of the number of genes differentiating the qualitative characters and their linkage relationships is essential to a correct interpretation. The data bearing on this point are given in table 1. The proportion of homozygous black glume: heterozygous black glume: homozygous white glume segregates was 252: 504: 249; the proportion of homozygous deficiens: heterozygous deficiens: homozygous vulgare was 241: 509: 255; and the proportion of homozygous for normal habit of growth: those heterozygous for normal habit of growth: those homozygous for brachytic habit of growth was 269: 488: 248. These figures are based on the segregation as determined by classification of the F_2 and a progeny test in the F_3 generation. The expected ratio for all three characters based on the hypothesis that the contrasted characters are differentiated by 1 factor pair is 251.25: 502.50: 251.25. The P values obtained by applying the χ^2 test were found to lie between .98 and .95, .80 and .70 and .50 and .30 for color of glume, type of spike and habit of growth, respectively. It is apparent that the three qualitative characters are differentiated by a single factor pair.

TABLE 1

Linkage relationships between three qualitative characters as determined by partitioning χ^2 for goodness of fit into its components.

TYPE OF SPIKE *vs.* COLOR OF GLUMES		HABIT OF GROWTH *vs* COLOR OF GLUMES		TYPE OF SPIKE *vs.* HABIT OF GROWTH		EXPECTED IF INDEPENDENT
GENOTYPE	OBTAINED	GENOTYPE	OBTAINED	GENOTYPE	OBTAINED	
VVBB	56	*BrBrBB*	70	*VVBrBr*	78	62.81
VVBb	115	*BrBrBb*	128	*VVBrbr*	109	125 62
VVbb	70	*BrBrbb*	71	*VVbrbr*	54	62 81
VvBB	134	*BrbrBB*	118	*VvBrBr*	130	125.63
VvBb	256	*BrbrBb*	257	*VvBrbr*	249	251.25
Vvbb	119	*Brbrbb*	113	*Vvbrbr*	130	125.63
vvBB	62	*brbrBB*	64	*vvBrBr*	61	62.81
vvBb	133	*brbrBb*	119	*vvBrbr*	130	125.63
vvbb	60	*brbrbb*	65	*vvbrbr*	64	62.81
P between .50 and 30		P between .80 and .70		P between .30 and .20		

The data concerning the linkage relationships of these three qualitative characters are given in table 1 also. The P values of χ^2 for independent inheritance for type of spike and color of glume, habit of growth and color of glume and type of spike and habit of growth lie between .50 and .30, .80 and .70, and .30 and .20 respectively. It may be concluded that the genes differentiating the characters under consideration are located in different chromosomes. That color of glumes and type of spike are independently inherited has been shown by previous workers. For a review of literature on linkage relationships see DAANE (1931). It is apparent that any effect upon yield, number of spikes, height, or length of awn that may be found to be associated with any of these pairs of alleles will be independent, as far as linkage relationships are concerned, from the effect that any different pair of alleles might be exerting.

Genes Associated With Color of Glumes

The means of weight of seed, spikes per plant, height of plant, and length of awn for the three different genotypes are given in table 2. The genotype was determined by segregation in the F_2 and a progeny test in the F_3 generation; whereas, the measurements are for the F_2 generation.

TABLE 2

The means of four quantitative characters measured in the F_2 generation of a cross between B1, and Brachytic, classified into phenotypes and genotypes according to color of glumes.

PHENOTYPES	GENOTYPES	WEIGHT OF SEEDS IN GRAMS	NUMBER OF SPIKES PER PLANT	HEIGHT OF PLANT IN INCHES	LENGTH OF AWN IN MM
White	(*bb*)	4.7	5.9	24.4	117.3
Black	(*Bb*)	5.0	6.0	24.6	119.6
Black	(*BB*)	4.7	5.8	24.2	117.8

If a t value of 1.960 which gives a P value of .05 is taken as statistically significant, the seed yield of the *Bb* segregates was significantly greater than either those of the genotype *BB* or *bb;* whereas, the weights of seed per plant for the *BB* and *bb* segregates were practically the same. The t values were 2.501, 2.491 and .148 respectively. The only other t values exceeding 1.960 were those for *Bb* and *bb* in comparing length of awn and *Bb* and *BB* in comparing height of plant. However it can be seen from table 2 that in every case the mean of the heterozygote exceeds those of the two homozygotes for the four characters. Even though the differences between the two homozygotes and the heterozygote are small, there seems to be little reason for questioning their biological significance. Since the differences between the two homozygotes are neither consistent nor have t values sufficiently large to closely approach a P. of .05, it can be concluded that *bb* and *BB* do not have a differential effect upon weight of seed, spikes per plant, height of plant, nor length of awn.

These results can be explained by JONES' (1917) hypothesis to account for heterosis. If this explanation is accepted then the increases noted for the heterozygote must be due to favorable, and at least partially dominant genes, some of which must be located on the same chromosome with

B and others on the homologous chromosome with *b*. Furthermore, it must follow from the results obtained, if we are to accept JONES' hypothesis, that in the homozygous condition the genes located in the same chromosome with *B* have a similar effect upon the characters under consideration as do the genes linked with *b* when they are in a homozygous condition.

Genes Associated with Type of Spike and Habits of Growth

The data showing the reaction of the genes upon the four quantitative characters are given in tables 3 and 4. Table 3 is designed to facilitate an analysis of the differences between the *vv* and *Vv* segregates, the *vv* and *VV* segregates, and the *Vv* and *VV* segregates. These differences are shown for plants of the genotypes ***BrBr***, ***Brbr***, and ***brbr***, making a total of nine comparisons for each of the four quantitative characters. Table 4 is the same as table 3 except that it is designed to facilitate an analysis of the differences between the ***BrBr*** and ***Brbr*** segregates, the ***BrBr*** and ***brbr*** segregates, and the ***Brbr*** and ***brbr*** segregates.

From Table 3, it can be seen that in every case the *vv* plants gave a larger yield of seed per plant than did the *Vv* or *VV* segregates and *Vv* segregates exceeded *VV* segregates. The lowest t value obtained for any of these comparisons was 5.495 which gives odds greater than 99:1 against the deviations noted being due to the errors of random sampling. *Vv* exceeded *vv* plants among the ***BrBr*** and ***Brbr*** segregates for number of spikes per plant, height of plant, and length of awn and the t values for the differences had a P value of less than .01 in all cases. Likewise, the *Vv* plants exceeded the *vv* plants among the ***brbr*** progeny but the t values were less than the above; that for number of spikes per plant being .658, for height of plant 3.197, and for length of awn 1.601. It will be remembered that a value of 1.960 is necessary for a P value as low as .05. For spikes per plant, height of plant, and length of awn the *VV* segregates exceeded those possessing *vv* for the ***BrBr*** and ***Brbr*** genotypes, but the t values for this comparison in the ***BrBr*** genotypes were only .700 and 1.632 for number of spikes per plant and height of plant, respectively, and 2.467 for length of awn. The t values in the ***Brbr*** progeny for spikes per plant, height of plant, and length of awn were 2.207, 5.304 and 4.898 respectively. The reverse was true for the ***brbr*** segregates since the *vv* progeny exceeded the *VV* progeny. The t values were 4.268, 1.968 and 4.268 which are sufficiently large to give substantial odds against the differences noted being chance deviations due to the errors of random sampling.

Table 4 shows that in respect to weight of seed per plant ***Brbr*** exceeded ***BrBr*** in two of the three comparisons, namely, in the *VV* and *vv* segregates. The odds against the difference being due to the errors of random sampling in the first case were a trifle less than 99:1 and in the second

TABLE 3

The comparative effect upon four quantitative characters of genes differentiating and associated in inheritance with type of spike.

PHENOTYPE AND GENOTYPE	WEIGHT OF SEED PER PLANT IN GRAMS			SPIKES PER PLANT			HEIGHT OF PLANT IN INCHES			LENGTH OF AWN IN MILLIMETERS		
	WEIGHT		DIFFERENCE	NUMBER		DIFFERENCE	HEIGHT		DIFFERENCE	LENGTH		DIFFERENCE
Vulgare (*vv*) vs. deficiens (*Vv*)												
Normal (*BrBr*)	7.3	5.1	2.22	5.7	7.4	−1.72	25.9	27.4	−1.54	127.3	143.8	−16.58
Normal (*Brbr*)	8.0	5.0	2.98	5.6	6.6	−.94	25.3	27.3	−2.08	126.3	142.7	−16.42
Brachytic (*brbr*)	4.5	2.6	1.88	4.8	4.9	−.13*	17.2	18.3	−1.03	62.7	64.7	− 1.95*
Vulgare (*vv*) vs. deficiens (*VV*)												
Normal (*BrBr*)	7.3	3.9	3.44	5.7	5.9	− .21*	25.9	26.5	− .64*	127.3	134.8	− 7.50†
Normal (*Brbr*)	8.0	4.2	3.74	5.6	6.0	− .39*	25.3	26.7	−1.41	126.3	135.5	− 9.20
Brachytic (*brbr*)	4.5	1.7	2.74	4.8	3.8	.94	17.2	16.5	.68†	62.7	50.0	6.68
Deficiens (*Vv*) vs. deficiens (*VV*)												
Normal (*BrBr*)	5.1	3.9	1.22	7.4	5.9	1.51	27.4	26.5	.90	143.8	134.8	9.08
Normal (*Brbr*)	5.0	4.2	.76	6.6	6.0	.55	27.3	26.7	.67†	142.7	135.5	7.22
Brachytic (*brbr*)	2.6	1.7	.86	4.9	3.8	1.07	18.3	16.5	1.71	64.7	56.0	8.63
B1 parent (*VVBrBr*) vs. F$_2$ (*VVBrBr*)	1.9	3.9	−2.00	3.7	5.9	−2.2	25.1	26.5	−1.4	137.8	134.8	3.00*
Brachytic parent (*vvbrbr*) vs. F$_2$ (*vvbrbr*)	4.00	4.5	− .50*	4.7	4.8	− .10*	16.1	17.2	−1.1	71.0	62.7	8.3
B1 parent (*VVBrBr*) vs. B1 parent (*VVBrBr*)	1.9	1.9	.00	3.8	3.7	.10*	25.6	25.1	.5*	141.6	137.8	3.8*

* t test gives P>.05.

† t test gives P<.05 but>.01.

TABLE 4

The comparative effect upon four quantitative characters of genes differentiating and associated in inheritance with habit of growth.

PHENOTYPE AND GENOTYPE	WEIGHT OF SEED PER PLANT IN GRAMS			SPIKES PER PLANT			HEIGHT OF PLANT IN INCHES			LENGTH OF AWN IN MILLIMETERS		
	WEIGHT		DIFFERENCE	NUMBER		DIFFERENCE	HEIGHT		DIFFERENCE	LENGTH		DIFFERENCE
Normal (*BrBr*) vs. normal (*Brbr*)												
Vulgare (*vv*)	7.3	8.0	− 67*	5.7	5.6	.05*	25.9	25.3	.60*	127.3	126.3	.95*
Deficiens (*Vv*)	5.1	5.0	.09*	7.4	6.6	.83	27.4	27.3	.06*	143.8	142.7	1.11*
Deficiens (*VV*)	3.9	4.2	−.37†	5.9	6.0	−.13*	26.5	26.7	−.17*	134.8	135.5	−.75*
Normal (*BrBr*) vs. brachytic (*brbr*)												
Vulgare (*vv*)	7.3	4.5	2.82	5.7	4.8	.90	25.9	17.2	8.63	127.3	62.7	64.56
Deficiens (*Vv*)	5.1	2.6	2.48	7.4	4.9	2.49	27.4	18 3	9.14	143.8	64.7	79.19
Deficiens (*VV*)	3.9	1.7	2.12	5.9	3.8	2.05	26.5	16.5	9.95	134.8	56.0	78.74
Normal (*Brbr*) vs. brachytic (*brbr*)												
Vulgare (*vv*)	8.0	4.5	3.49	5.6	4 8	.85	25.3	17.2	8.03	126.3	62.7	63.61
Deficiens (*Vv*)	5.0	2.6	2.39	6.6	4.9	1.66	27.3	18.3	9.08	142.7	64.7	78.08
Deficiens (*VV*)	4.2	1.7	2.49	6.0	3.8	2.18	26.7	16.5	10.12	135.5	56.0	79.49

* t test gives $P > .05$.

† t test gives $P < .05$ but $> .01$.

case were 9:1. It seems reasonably safe to conclude that the *Brbr* segregates in the *VV* plants possessed a somewhat larger weight of seed per plant than did the *BrBr* segregates. In every case and for all four characters, the *BrBr* and *Brbr* segregates exceeded the *brbr* plants and in no comparison were the odds against the differences being due to the errors of random sampling less than 99:1.

THE NATURE OF THE INTERACTION BETWEEN THE NON-ALLELIC GENES

The nature of the interaction between the non-allelic genes is of particular interest, as it is of fundamental importance to know whether the favorable growth genes give the same increase over the less favorable growth genes in all genotypes. To facilitate the study of this problem, the data in table 5 were compiled. This table gives the interactions between the genes differentiating and associated by linkage with type of spike and those differentiating and associated by linkage with habit of growth. A brief statement concerning the formulation of this table will help to clarify the discussion which follows. For illustration consider the interaction *vv* vs. *Vv* and *BrBr* vs. *Brbr*. The information given is whether the difference between *vv* and *Vv* in combination with *BrBr* is the same as in combination with *Brbr*. This may be tested by comparing the two differences. Thus the difference (*vvBrBr* – *VvBrBr*) – (*vvBrbr* – *VvBrbr*) for weight of seed is .76 grams, as can be seen from table 5 in which these differences between two differences are listed together with the t values for testing their significance. If the difference (*vvBrBr* – *VvBrBr*) – (*vvBrbr* – *VvBrbr*) is statistically significant, then it must necessarily follow that the difference

TABLE 5

The interactions between those genes differentiating and associated in inheritance with type of spike and habit of growth as regards their effect upon four quantitative characters.

INTERACTION	WEIGHT OF SEED DIFFERENCE		SPIKES PER PLANT DIFFERENCE		HEIGHT OF PLANT DIFFERENCE		LENGTH OF AWN DIFFERENCE	
	GRAMS	t	NO	t	IN	t	MM	t
Vulgare (*vv*) vs. deficiens (*Vv*) and								
Normal (*BrBr*) vs. normal (*Brbr*)	.76	2.183	78	2.693	54	1.195	16	058
Normal (*BrBr*) vs. brachytic (*brbr*)	34	.092	1.59	4.093	51	1.030	14.63	5.442
Normal (*Brbr*) vs. brachytic (*brbr*)	1.10	3.543	.81	3.039	1.05	2.446	14.47	6.227
Vulgare (*vv*) vs. deficiens (*VV*) and								
Normal (*BrBr*) vs. normal (*Brbr*)	30	697	18	.553	77	1.624	1.70	.503
Normal (*BrBr*) vs. brachytic (*brbr*)	70	1.627	–1.15	3.000	–1.32	2.484	–14.18	3.040
Normal (*Brbr*) vs. brachytic (*brbr*)	1.00	2.418	–1.33	4.503	–2.09	4.476	–15.88	5.486
Deficiens (*Vv*) vs. deficiens (*VV*) and								
Normal (*BrBr*) vs. normal (*Brbr*)	46	2.143	.96	3.676	.23	.542	1.86	.697
Normal (*BrBr*) vs. brachytic (*brbr*)	36	1.742	.44	1.524	.81	1.631	.45	.160
Normal (*Brbr*) vs. brachytic (*brbr*)	.10	469	.52	1.966	1.04	2.317	1.41	.581

(*vvBrBr*−*vvBrbr*)−(*VvBrBr*−*VvBrbr*) must also be statistically significant, as the final figures are identical. The reason for this is apparent as in both cases we are dealing with the same interaction stated differently. The former statement emphasises the *Vv* genes whereas the latter places the emphasis on the *Brbr* genes. In the discussion of the interactions in this article, for the sake of clarity emphasis will be placed on the *Vv* genes.

From the t values in table 5, it can be readily determined which interactions are statistically significant. The following interactions for weight of seed have t values larger than 1.960; *vv* vs. *Vv* and *BrBr* vs. *Brbr*, *vv* vs. *Vv* and *Brbr* vs. *brbr*, *vv* vs. *Vv* and *Brbr* vs. *brbr*, *vv* vs. *VV* and *Brbr* vs. *brbr*, and *Vv* vs. *VV* and *Brbr* vs. *BrBr*. Considering spikes per plant, only two of the nine interactions have t values lower than 1.960; whereas, both height of plant and length of awn have five t values below this figure. These data are conclusive in showing that the interactions between the non-allelic genes are of such a nature that the more favorable growth factors do not give the same increase over the less favorable growth factors in all genotypes.

The nature of this interaction as determined by which genotype induces larger increases can be determined from the data in tables 3, 4, and 5. Table 3 shows that *vv* plants combined with a high producing genotype are favored more than are *Vv* or *VV* plants, as concerns weight of seed per plant. For example, the difference between *vv* and *Vv* plants in the *BrBr* genotype is 2.22, in *Brbr* is 2.98, and in *brbr* is only 1.88. Likewise, the difference between *vv* and *VV* plants in the *BrBr* genotype is 3.44, in *Brbr* is 3.74, and in *brbr* is only 2.74 grams. From the t tests of the interactions given in table 5, it can be seen that 2.98 is significantly different from 2.22 and 1.88 and that 3.74 is significantly different from 2.74. Table 3 shows that the difference between *Vv* and *VV* plants was greater in the *BrBr* genotype than it was in *Brbr* or *brbr* and the t test for the interactions listed in table 5 show that this difference involving the *BrBr* and *Brbr* genotypes is probably statistically significant. Therefore, in this study, it is evident that the genes favorable to high plant yields when transferred from a low to a high yielding genotype are, in comparison with their alleles and in absolute values, still more favorable to plant growth. The only exceptions to this were the difference between the *Vv* and *VV* plants in the *Brbr* genotype in comparison with the difference between the *Vv* plants and the *VV* plants in the *brbr* genotype. The differences were obtained from means of 5.0 and 4.2 grams in comparison with 2.6 and 1.7 grams. In this case the smaller means did not give the smaller difference; that is, the *Vv* plants in comparison with the *VV* plants did not give an increased difference in the higher yielding genotypes.

It remains to be seen whether the same general conclusions can be drawn for the other three quantitative characters studied. In making the comparison between *vv* and *Vv* plants for the *BrBr* and *Brbr* genotypes. it is necessary to know whether the latter two genotypes differ. The only statistically significant difference between these two genotypes, as can be seen from table 4, was for spikes per plant in the *Vv* genotype. Then, if the more favorable genes give still greater increases when in the higher tillering genotypes, it would be expected that the difference between *vv* plants and *Vv* plants would be still greater in the *BrBr* genotype than the same comparison in the *Brbr* genotype. That such actually was the case can be seen from table 3, and that the difference between the two differences of −1.72 and −.94 was significant can be seen from table 5. The t value is larger than necessary to give odds of 99:1 against the deviation of .78 being due to the errors of random sampling. Likewise, according to the hypothesis that the genes more favorable to growth give greater increases over those less favorable in combination with genes also favorable to growth, the difference between *vv* and *Vv* plants of the genotypes *BrBr* and *Brbr* should be greater than the difference between *vv* and *Vv* plants in the *brbr* genotype. Such was found to be the case for all characters (see table 3) and with the exception of the comparison between the differences involving *vv* and *Vv* in the *BrBr* and *brbr* genotypes for height of plant the differences were statistically significant.

In comparing the *vv* and *VV* plants the *BrBr* and *Brbr* genotypes may be omitted as the latter two genotypes do not differ significantly in the three quantitative characters measured. The hypothesis that the differences would be still greater in the genotypes of higher values does not fit the data as regards the comparison between *vv* and *VV* plants in the genotypes *BrBr* and *Brbr* compared with *brbr*. For example, in the *brbr* genotype *vv* plants have a greater number of spikes per plant, a greater height and a longer awn; whereas, in the *Brbr* genotype the reverse is true and in the *BrBr* genotype the only significant difference is for length of awn and it is in favor of the *VV* plants. As regards the comparison between *Vv* and *VV* plants and involving the three characters spikes per plant, height of plant, and length of awn, the only consistent difference seems to be that in every case the *Brbr* genotype gave lower differences (table 3) than did the *BrBr* and *brbr* genotypes. The differences noted for the interaction *Vv* vs. *VV* and *BrBr* vs. *Brbr* for number of spikes were statistically significant as were also the differences for the interaction *Vv* vs. *VV* and *Brbr* vs. *brbr* for number of spikes and height of plant. The differences are given in tables 3 and 4 and the interaction in table 5.

The regression coefficients of weight of seed on number of spikes give additional information concerning the nature of the interactions of the genes influencing yield of seed per plant. Table 6 shows that in every case the addition of a spike among the *vv* segregates gave a greater increased yield than did an addition of a spike among the *Vv* or *VV* segregates. Likewise an addition of a spike per plant gave a greater increase in yield among the *Vv* segregates than did the addition of a spike per plant among the *VV* segregates. The difference between these two genotypes is statistically significant in two out of the three comparisons. Turning to the different combinations of *Br* and *br*, the *Brbr* plants gave a greater increased yield for each additional spike per plant than did the *BrBr* plants, (see table 7). In only one case however, did the odds against the differences being due to the errors of random sampling exceed 19:1. In every case an additional spike gave a greater increase per plant in the *BrBr* segregates than was the case as regards the *brbr* segregates. Here again, only one of the differences was statistically significant but it was decidedly so. In all cases the *Brbr* segregates gave a greater increased yield per additional spike than was true for the *brbr* segregates and all the differences were statistically significant.

TABLE 6

The regression coefficients of weight of seed on number of spikes compared on the basis of genes associated in inheritance with type of spikes.

PHENOTYPE AND GENOTYPE	WEIGHT OF SEED PER PLANT ON NUMBER OF SPIKES			
	REGRESSION COEFFICIENTS		DIFFERENCE	t
	grams	*grams*	*grams*	
Vulgare (*vv*) vs. deficiens (*Vv*)				
Normal (*BrBr*)	1.03	.73	.30	3.121
Normal (*Brbr*)	1.40	.78	.62	8.448
Brachytic (*brbr*)	.82	.56	.26	2.555**
Vulgare (*vv*) vs. deficiens (*VV*)				
Normal (*BrBr*)	1.03	.63	.40	3.348
Normal (*Brbr*)	1.40	.69	.71	7.490
Brachytic (*brbr*)	.82	.51	.31	2.297**
Deficiens (*Vv*) vs. deficiens (*VV*)				
Normal (*BrBr*)	.73	.63	.10	1.995**
Normal (*Brbr*)	.78	.69	.09	1.942*
Brachytic (*brbr*)	.56	.51	.05	.915*
B1 parent (*VVBrBr*) vs. F_2 genotype (*VVBrBr*)	.53	.63	−.10	1.968**
Brachytic parent (*vvbrbr*) vs. F_2 genotype (*vvbrbr*)	.70	.82	−.12	.870*
B1 parent (*VVBrBr*) vs. B1 parent (*VVBrBr*)	.54	.53	.01	.142*

* t gives $P>.05$ ** t gives $P<.05$ but $>.01$.

It is of interest to know the manner of interaction that exists among the genes affecting yield. For example, do *vv* plants give the same increase per additional spike over *Vv* plants, whether in combination with *BrBr*, *Brbr*, or *brbr?* It is evident from table 6 that the increase is greater in combination with *Brbr* than when in combination with *BrBr* or *brbr*. The same is true for the comparison between the *vv* and *VV* plants. The t values for these interactions were found to exceed 1.96. It is evident that a difference in interaction of factors exists, and the only general conclusion to be drawn, and which does not hold for all cases, is that genes favorable to increased yield per additional spike give still greater absolute increased yields when in combination with other genes which also favor the development of this character.

TABLE 7

The regression coefficients of weight of seed on number of spikes compared on the basis of genes associated in inheritance with habit of growth.

PHENOTYPE AND GENOTYPE	WEIGHT OF SEED PER PLANT ON NUMBER OF SPIKES			
	REGRESSION COEFFICIENTS		DIFFERENCE	t
	grams	*grams*	*grams*	
Normal (*BrBr*) vs. Normal (*Brbr*)				
Vulgare (*vv*)	1.03	1.40	−.37	2.459**
Deficiens (*Vv*)	.73	.78	−.05	1.200*
Deficiens (*VV*)	.63	.69	−.06	1.260*
Normal (*BrBr*) vs. brachytic (*brbr*)				
Vulgare (*vv*)	1.03	.82	.21	.994*
Deficiens (*Vv*)	.73	.56	.17	3.934
Deficiens (*VV*)	.63	.51	.12	1.853*
Normal (*Brbr*) vs. brachytic (*brbr*)				
Vulgare (*vv*)	1.40	.82	.58	3.010
Deficiens (*Vv*)	.78	.56	.22	4.512
Deficiens (*VV*)	.69	.51	.18	3.089

* t gives $P > .05$ ** t gives $P < .05$ but $> .01$.

COMPARISON BETWEEN THE F_1 GENERATION, CERTAIN F_2 GENERATION GENOTYPES AND THE PARENTS

The comparison of the F_1 generation and parents and certain genotypes of the F_2 generation and parents provides information concerning the effect upon weight of seed per plant of genes not linked with the qualitative characters analyzed in this study (table 8). It will be remembered from the discussion of methods that due to the fact that F_1 and F_2 generations were grown some distance apart, although in the same series, a comparison between them must be made by use of the parents. The difference in yield between the F_1 generation *VvBrbr* and the B 1 parent *VVBrBr* was 4.1 grams; whereas, the difference in yield between the F_2 generation plants *VvBrbr* and the B 1 parent *VVBrBr* plants was 3.1 grams (table 8). Both of these differences have a P value of less than .01 and therefore, are undoubtedly statistically significant. The difference between the two differences also shows $P < .01$. This means that the difference between F_1 *VvBrbr* and the B 1 parent was greater than the difference between F_2

VvBrbr and B 1 parent. The same relationship held for the comparison between F_1 *VvBrbr* and F_2 *VvBrbr* plants with the Brachytic parent plants, but the difference between the two differences was not statistically significant. These data show that there must have been a reduction in weight of seed per plant between F_1 (*VvBrbr*) and F_2 (*VvBrbr*) plants. This reduction can be accounted for by partially dominant genes affecting yield and at least not closely linked with *Vv* or *Brbr*, probably the ones having the most effect being independently inherited.

The presence of these genes may be tested further by comparing the F_2 plants of the genotypes *VVBrBr* and *vvbrbr* with the parental plants of the same genotypes, respectively (see table 8). If there are no genes other than those closely linked with *Vv* and *Brbr* which affect yield and are segregating, the differences in yield between parental and F_2 plants of the corresponding genotype should not be statistically significant. Table 8 shows that the difference between F_2 plants *VVBrBr* and the B 1 parent *VVBrBr* is statistically significant, whereas that between the F_2 *vvbrbr* plants and the Brachytic parent *vvbrbr* is not. Also, the difference betwen the two differences is statistically significant. It is evident that the genes more favorable to high yield in the F_2 plants of the genotype *VVBrBr* than in F_2 plants of the genotype *vvbrbr* must have entered the cross from the Brachytic parent. There is no corresponding depressing effect on yield of grain per plant of the alleles of these genes entering the cross from the B 1 parent; if there were, the F_2 *vvbrbr* segregates should yield less than the Brachytic parent *vvbrbr*. Such was not the case. Again there is proof of a difference in the interaction of factors.

TABLE 8

Weight of seed per plant of the parents compared with weight of seed per plant of the F_1 and of certain F_2 genotypes.

COMPARISON	N	WEIGHT OF SEED PER PLANT IN GRAMS			
		WEIGHT		DIFFERENCE	t
F_1 (*VvBrbr*) vs. B1 parent (*VVBrBr*)	57 and 50	5.6	1.5	4.1	7.930
F_1 (*VvBrbr*) vs. brachytic parent (*vvbrbr*)	57 and 60	5.6	4.3	1.3	2.012**
F_2 (*VvBrbr*) vs. B1 parent (*VVBrBr*)	249 and 266	5.0	1.9	3.1	34.171
F_2 (*VvBrbr*) vs. brachytic parent (*vvbrbr*)	249 and 63	5.0	4.0	1.0	3.229
F_2 (*VVBrBr*) vs. B1 parent (*VVBrBr*)	78 and 266	3.9	1.9	2.0	28.189
F_2 (*vvbrbr*) vs. brachytic parent (*vvbrbr*)	64 and 63	4.5	4.0	.5	.761*
F_1 (*VvBrbr*) vs. B1 parent (*VVBrBr*) and F_2 (*VvBrBr*) vs. B1 parent (*VVBrBr*)				1.0	4.148
F_2 (*VVBrBr*) vs. B1 parent (*VVBrBr*) and F_2 (*vvbrbr*) vs. brachytic parent (*vvbrbr*)				1.5	5.807

* t test gives $P > .05$.
** t gives $P < .05$ but $> .01$.

THE VARIANCES OF THE DIFFERENT GENOTYPES AND PARENTS

When studying quantitative characters, it has been the practice of geneticists to use homozygous material as a measure of the environmental variability. By the analysis of variance, it is possible to divide the total variability into that due to the genes isolated in the population being studied and that due to residual genetic variability plus environmental variability. In certain cases it is desirable to determine the residual genetic variability. If the parents can be used to measure the environmental variability, it can be accomplished readily by analyzing the variability into that due to genotypes and within genotypes. The variability within genotypes minus the environmental variability which has been measured by the parents would leave the residual genic variability. This method has been used by RASMUSSON (1935).

TABLE 9

The means and non adjusted variances for the different phenotypes and genotypes.

PHENOTYPE	GENOTYPE	WEIGHT OF SEED IN GRAMS		NUMBER OF SPIKES PER PLANT		HEIGHT OF PLANT IN IN		LENGTH OF AWN IN MM.	
		MEAN	VARIANCE	MEAN	VARIANCE	MEAN	VARIANCE	MEAN	VARIANCE
Vulgare normal	(*vvBrbr*)	8.0	19.280	5.0	6.562	25.3	5.705	120.3	237.516
Vulgare normal	(*vvBrBr*)	7.3	16.625	5.7	8.157	25.0	6.794	127.3	384.996
Deficiens normal	(*VvBrBr*)	5.1	7.744	7.4	12.240	27.4	7.323	143.8	265.903
Deficiens normal	(*VvBrbr*)	5.0	7.518	6.6	9.578	27.3	7.067	142.7	226.634
Vulgare Brachytic	(*vvbrbr*)	3.5	5.224	4.8	2.880	17.2	3.824	62.7	73.577
Deficiens normal	(*VVBrbr*)	4.2	4.046	6.0	8.194	26.7	4.726	135.5	230.844
Deficiens normal	(*VVBrBr*)	3.0	5.350	5.0	10.467	26.5	5.837	134.8	320.472
Deficiens Brachytic	(*Vvbrbr*)	2.6	2.588	4.9	6.106	18.3	5.834	64.7	75.796
Deficiens Brachytic	(*VVbrbr*)	1.7	1.560	3.8	4.858	16.5	5.536	56.0	121.754
B 1 Parent	(*VVBrBr*)	1.9	1.668	3.7	4.553	25.1	9.224	137.8	241.487
Brachytic Parent	(*vvbrbr*)	4.0	7.693	1.7	7.426	16.1	2.027	71.0	47.822
B 1 Parent	(*VVBrBr*)	1.9	1.690	3.8	4.641	25.6	6.617	141.6	163.416

Table 9 gives the variances and means for the different genotypes and parents. It should be recalled that the variances found for the Brachytic and B 1 parents listed in the last two rows of table 9 are not directly comparable with these given for the F_2 genotypes and B 1 parent, as they were grown in rows at the end of the series, whereas the data from the nine B 1 families, distributed at random with the twenty F_2 families would be comparable with the data from the F_2. The Brachytic parental data would be comparable with the F_2 data by means of the B 1 parent.

As regards weight of seed per plant, it is apparent that all genotypes are not equally variable. The genotypes with the larger means also have the larger variances. This is equally true for the parents. The relationship is not so close for the number of spikes per plant, height, and length of awn, but it does exist.

The comparison between the parents and F_1 plants furnishes additional evidence. In this study the mean weight per plant of the B 1 parent was 1.5 grams and the variance 1.379, for the Brachytic parent was 4.3 grams and the variance 6.219 and for the F_1 the mean was 5.6 grams and the variance 10.526.

It seems advisable to determine whether genotypes having means of similar magnitude have variances of different magnitudes. The means of the *Brbr* segregates for all four quantitative characters are similar to the means of the *BrBr* segregates. To ascertain whether differences in variability existed, the variances were obtained for all of the *Brbr* and *BrBr* segregates. FISHER's Z test (1934, p. 216) was applied. It was found that the variance for the *BrBr* plants was larger than the variance for the *Brbr* segregates for number of spikes and length of awn. The values obtained by dividing Z by its standard error were 2.260 and 2.624 respectively. It appears that all genotypes do not have the same variance even though their means may be of similar magnitude.

These results make it apparent that in studies such as this, erroneous conclusions are likely to be drawn in estimating the residual genic variability, by use of parental data as an absolute measure of environmental variation, as the amount of variation due to the environment is not the same for all genotypes.

THE NATURE OF THE INTERACTION BETWEEN THE GENES AFFECTING WEIGHT OF SEED PER PLANT AND THE ENVIRONMENT

It was found that in general the genes more favorable to higher yields of seed per plant gave still higher absolute yields in comparison with their alleles when in combination with high yielding genotypes than when in combination with low yielding genotypes. This raises the question as to whether the same relationship might not exist in a comparison involving different environments.

To obtain evidence the population for each of the nine genotypes was divided into three levels of yield by the use of the normal curve. The levels thus established within reasonable limits gave the same number of individuals in each class. This method of division does not eliminate the residual genic variability as genes not closely linked and those inherited independently of those identified in this study would have an effect as well as environmental conditions. Thus some of the plants falling in the upper 1/3 may be in that range partially because of favorable genes. An estimate of the importance of the effect of the residual genic variability can be obtained by a comparison of the weight of seed per plant for the parents and the F_2 segregates of the same genotype. The comparison is given in table 3. The B 1 parent (*VVBrBr*) yielded 2 grams less than the F_2 plants of the genotype *VVBrBr* and the Brachytic parent (*vvbrbr*) yielded .5 of a gram less than the F_2 plants of the genotype *vvbrbr*. From table 10, it can be seen that the range in yields of any one genotype for the different levels is considerably greater than the above. Therefore, from these data it appears that the environment played the most important part in determining into which level of yield a plant of a given genotype would fall.

The three levels of weight of seed per plant are listed in table 10. The difference between combinations of *Vv* and the differences between the combinations of *Brbr* are listed in table 11. As in all the previous studies these comparisons include the effect upon weight of seed per plant of the genes linked with *Vv* and *Brbr* as well as the effect of these genes themselves. The completeness with which the effect of the linked genes is measured depends of course upon the closeness of the linkage relationship. It is apparent from table 11 that, with the possible exception of *BrBr* compared with *Brbr* in combination with *VV*, the more favorable the environmental conditions the greater in absolute values the spread between the genes more favorable to yield and those less favorable to yield. Also, as noted from the previous data, the differences are greater for the higher yielding genotypes.

TABLE 10

Weight of seed per plant of the different genotypes for three levels of yield

PHENOTYPE	GENOTYPE	LEVEL OF YIELD		
		UPPER	MIDDLE	LOWER
Vulgare normal	(*vvBrBr*)	12.1	7.2	3.2
Vulgare normal	(*vvBrbr*)	13.5	7.9	4.1
Vulgare brachytic	(*vvbrbr*)	6.9	4.4	2.1
Deficiens normal	(*VvBrBr*)	8.7	5.2	2.3
Deficiens normal	(*VvBrbr*)	8.1	4.9	2.3
Deficiens brachytic	(*Vvbrbr*)	4.5	2.4	1.1
Deficiens normal	(*VVBrBr*)	6.9	3.9	1.4
Deficiens normal	(*VVBrbr*)	6.8	4.1	2.2
Deficiens brachytic	(*VVbrbr*)	3.3	1.6	.7

DISCUSSION AND CONCLUSIONS

Recently geneticists have become interested in the nature of the interaction of factors governing the inheritance of quantitative characters. Of interest in this respect is RASMUSSON'S (1935) interaction hypothesis which assumes "that the effect of each factor on the genotype is dependent upon all the other factors present, the visible effect of a certain factor being smaller the greater the number of factors acting in the same direction."

RASMUSSON found support for his hypothesis in a study on the interaction of factors governing early and late maturity in Pisum. POWERS (1934) in studying the inheritance of habit of growth in Triticum obtained results which would support this hypothesis also. However, the nature of the interaction of the factors affecting weight of seed per plant, number of spikes per plant, height of plant and length of awn was, generally speaking, quite the reverse of that expected on the above hypothesis in that the effect of certain factors was not smaller the greater the number acting in a certain direction.

TABLE 11

Differences in weight of seed per plant for three levels of yield.

GENOTYPE	LEVELS OF YIELD		
	UPPER	MIDDLE	LOWER
vv vs. *Vv* (*BrBr*)	3.4	2.0	.9
vv vs. *VV* (*BrBr*)	5.2	3.3	1.8
Vv vs *VV* (*BrBr*)	1.8	1.3	.9
vv vs *Vv* (*Brbr*)	5.4	3.0	1.8
vv vs. *VV* (*Brbr*)	6.7	3.8	1.9
Vv vs. *VV* (*Brbr*)	1.3	.8	.1
vv vs. *Vv* (*brbr*)	2.4	2.0	1.0
vv vs. *VV* (*brbr*)	3.6	2.8	1.4
Vv vs. *VV* (*brbr*)	1.2	.8	.4
BrBr vs. *Brbr* (*vv*)	−1.4	−.7	−.9
BrBr vs. *brbr* (*vv*)	5.2	2.8	1.1
Brbr vs. *brbr* (*vv*)	6.6	3.5	2.0
BrBr vs. *Brbr* (*Vv*)	.6	.3	.0
BrBr vs. *brbr* (*Vv*)	4.2	2.8	1.2
Brbr vs. *brbr* (*Vv*)	3.6	2.5	1.2
BrBr vs. *Brbr* (*VV*)	.1	−.2	−.8
BrBr vs. *brbr* (*VV*)	3.6	2.3	.7
Brbr vs. *brbr* (*VV*)	3.5	2.5	1.5

The logical conclusion to be drawn from these data is that the nature of the interaction of the genes affecting the quantitative characters is sufficiently variable to render any hypothesis of doubtful value as a means of prediction. From our more extensive knowledge of the interaction of factors governing the inheritance of quantitative characters it is not surprising that this is the case. In fact, BRINK (1934) found that the interaction of factors governing anthocyanin plant colors in maize was such that plants of the genotypes *AbPl* and *ABpl* averaged higher in dry weight of ears per plant than did plants of the genotypes *ABPl* and *Abpl*. It seems from these results and others reported here that the nature of the interaction of factors affecting quantitative characters is sufficiently variable to require extensive genetic studies involving a large variety of characters and organisms before a hypothesis of much value for prediction purposes can be formulated and then it may be expected to be rather limited in application.

EAST (1935) develops a concept concerning genes that is useful in the interpretation of any studies on quantitative inheritance. He divides gene mutations into two broad classes: physiological defectives and physiological non-defectives. These are discussed in connection with the bearing that they have upon evolution. Physiological defective gene mutations comprise the great bulk of those found in the genetic laboratory, usually are recessive to the wild type and cause restrictions in the physiological processes in which they are involved. This would mean that they may have both quantitative and qualitative effects and are easily detectable. Because of the comparative ease with which they are detected the effect must be pronounced. The non-defective gene mutations are frequent in nature but are difficult to detect individually and may show either an approach to dominance or to recessiveness. The effect of any one gene resulting as a non-defective mutation must be small.

The importance of this conception of the two broad types of gene mutations for a better understanding of the genes differentiating quantitative characters is obvious. It can be expected that the characters depending upon physiological defective genes for their expression will be differentiated from their alleles by comparatively few factor pairs; whereas, larger differences involving non-defective genes would be expected to be differentiated by a large number of gene pairs, because as is pointed out by EAST (1935) the effect of any one pair of non-defective genes is small.

The results from the present study offer some evidence for the conception that genes having an effect upon quantitative characters may be grouped into the classes noted above. The *brbr* genes which cause brachytic habit of growth would certainly be classed as physiological defective, as would the *VV* genes which produce *deficiens* type of spike. Plants possessing *VV* genes do not have kernels developed in the rudimentary lateral florets. The heterozygote *Vv* approaches the *deficiens* parent in that no lateral grains are produced, but it is distinguishable from the *VV* homozygote in that the lateral florets although rudimentary are noticeably developed. Undoubtedly the effects of the *Brbr* and *Vv* genes are both quantitative and qualitative. The genes affecting the four quantitative characters and associated with color of glume are of the non-defective type. In heterosis at least two factor pairs are necessary to account for the increases noted in the heterozygote, since the differences between the two homozygotes are not statistically significant (table 2). It seems highly probable that even larger numbers of factor pairs than these are [illegible]ing to produce the results obtained. For all four characters the Vv pla[illegible]eeded the VV plants. Again the most probable explanation seems to [illegible]t a number of partially dominant linked factors are responsible for [illegible]fferences between the two genotypes. As regards the genes associa[illegible]th color of glumes, the effect of any one factor pair must necessarily [illegible]all, and as EAST (1935) has pointed out such small differences are di[illegible]to detect.

ROBERTSON AND AUSTIN (1936) studied homozygous an[illegible]erozygous green plants of *Hordeum vulgare* from families segregating [illegible] the single factor pairs X_cx_c and A_ca_c and found statistically significant [illegible]ences in favor of the heterozygous plants for the following charact[illegible] average length of head, total number of grains per plant, and total wei[illegible] of grain per plant. Again the differences were small and may be due to [illegible] of physiologically non-defective genes. The residual genic variab[illegible] noted in the study reported in this article is due probably to factors o[illegible] same nature (non-defective) as the writer failed to find any qualitativ[illegible] pression of them. That these genes did not react alike in all genoty[illegible] was shown by the fact that greater increases in weight of seed per pla[illegible]ere obtained when combined in plants of the genotype $VVBrBr$ tha[illegible]n combined in plants of the genotype $vvbrbr$. These results show that [illegible] groups of genes—physiological defective and non-defective—do not [illegible]sarily react alike in all genotypes.

It should be pointed out that the terms physiological defective and no[illegible]defective are used by EAST in reference to gene mutations, but it is app[illegible]ent that his theorems are important in a study of the inheritance of quantitative characters. Undoubtedly these two classes grade into each other and exceptions exist, but this does not vitiate the value of such a classificatio[illegible] It is apparent that the above conception is very useful to the workers in the field of applied genetics. Rapid strides can be made by the breeder when dealing with the physiological defective genes, whereas, when non-defective genes are involved, the advantage gained by recombining several desirable factor pairs may be expected to be small and the progeny possessing them difficult to select because of the limited number in large populations. This does not mean that the non-defective genes are not important in a breeding program, but only that they are more difficult to work with.

SUMMARY

1. A method involving the analysis of variance and co-variance was used in reducing the data.

2. The nature of the interactions of genes was such that no general rule could be drawn. However, with one exception, which was not well estab-

lished statistically, the genes favorable to high weight of seed per plant gave as great or greater differences over their alleles in combination with genes for higher yield than they did in combination with genes for lower yield. The same general behavior was noted for the genes affecting number of spikes per plant, height of plant, and length of awn, but cases were found in which plants of a given genotype surpassed plants possessing their alleles in some combinations and were surpassed by plants possessing identi in other combinations.

genic variability not associated with any of the three chromosor ps identified by genes having qualitative effects was found to giv ater increased weight of seed per plant when in combination with *VV* than when in combination with *vvbrbr*. Here, again, is proof of a dif e in the interaction of factors.

was found that genotypes affecting the same character may have di t variances even though their means may be of similar magnitude.

t was found that the more favorable the environmental conditions th eater in absolute values would be the spread between the genes more f able to yield and those less favorable to yield.

The nature of the interaction of the factors affecting weight of seed plant, number of spikes per plant, height of plant, and length of awn s generally speaking quite the reverse of RASMUSSON's interaction-hypothesis which assumes that the visible effect of a certain factor is smaller the greater the number of factors acting in the same direction.

7. The genes affecting the four quantitative characters are grouped into physiological defective and non-defective according to EAST's (1935) terminology and the data offer some evidence in favor of his conception as to the nature of the effects of these genes. Both classes of genes showed that they necessarily did not give the same type of interaction in all genotypes.

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GENETIC STUDIES ON SELECTIVE SEGREGATION OF CHROMOSOMES IN SCIARA COPROPHILA LINTNER

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INTRODUCTION

THE unusual behavior of the chromosomes in Sciara has been studied in considerable detail both cytologically and genetically. Although the main facts regarding the distribution and behavior of the chromosomes (as revealed by cytological examination), have been known for some time, the genetic data have been incomplete because of the paucity of mutant characters with which to follow the history of the individual chromosomes in any one species. The present paper is designed to supply additional genetic data derived from an intensive study of *Sciara coprophila* Lintner, over a period of five years. Because of the great difficulty of securing mutant characters in Sciara, only a few have been obtained, even with the aid of X-rays, and some of these are unsatisfactory. Even the latter have been included, however, in order to make the account as complete as possible. The main results of this study were presented by SMITH (1932) but publication of the present account has been delayed in the hope of securing more and better characters—a hope that has not been realized.

Previous genetic studies on this species have dealt with one pair of autosomes (METZ 1927, truncate wings), and the sex chromosomes (METZ, ULLIAN, SCHMUCK, SMITH 1929 31). The new characters considered here make it possible to present the essential facts concerning the behavior of the remaining pairs of autosomes, as well as additional data concerning the first pair.

Before analyzing the genetic results in detail, it is necessary to review briefly certain aspects of chromosome behavior in Sciara. One feature of importance is the difference in chromosome number between the two sexes, and between the soma and germ line in each sex (METZ 1931). These differences are brought about by a process of chromosome elimination during cleavage in the developing egg (DUBOIS 1932). The typical number of chromosomes in somatic groups is eight in the female (one pair of V's, three pairs of rods); and seven in the male (one pair of V's, two pairs of rods, one single rod). These are shown in figure 1a and 1b. The male group is similar to the female group but lacks one rod, presumably a sex chromosome. There are present in the germ-line of both sexes, one, two, or three additional chromosomes which are longer and thicker than the ordinary chromosomes. These are termed the "limited" chromosomes (fig. 1c), (Metz 1931, Metz and Schmuck 1931). Since they are found only in the germ-line, they cannot be studied by means of somatic characters and consequently will not be considered in this account. The evidence indicates, however, that these chromosomes are relatively empty of genes (Metz 1929), and that they are not true sex chromosomes.

Another phenomenon of special significance in the present study is the occurrence of a "monocentric" mitosis at the first spermatocyte division (Metz 1926, Metz, Moses and Hoppe 1926, Metz 1933). This mitosis is accompanied by a selective segregation of chromosomes. During prophase at this division, the chromosomes are distributed at random throughout the nucleus. Although the chromosomes are present in pairs, no evidence of synapsis has been observed at any stage of spermatogenesis. A half spindle is formed with a single pole to which all the chromosomes are attached by "spindle fibres." Subsequently, without forming an equatorial plate, they move directly into anaphase. Both of the "limited" chromosomes go regularly toward the pole, but the others segregate in such a way that one member of each pair goes toward the visible pole and the other away from it, despite the fact that the "spindle fibres" of all extend toward the pole.

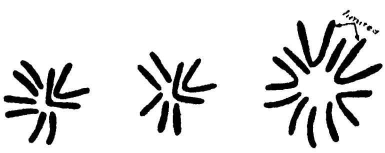

Figure 1. Diagrams showing chromosome groups in (a) female somatic cell, (b) male somatic cell, (c) germ-line of both sexes with "limited" chromosomes. In somatic cells the chromosomes are associated in pairs, while in oogonia and spermatogonia they are not.

When the four retreating chromosomes reach the periphery of the cell, they are deflected in their course, as if the cell wall were a mechanical barrier, and eventually they come together in a group opposite the pole. Later they are extruded in a bud resembling a polar body, and take no further part in development. From the cytological evidence, it is clear that the chromosomes are distributed here in a definite and regular way, so that one group is left in the functional cell, and the other is discarded. The question now arises as to which chromosomes are retained and which are cast off, and what is the nature of the forces operating to produce this result.

The first evidence bearing on the problem was obtained through a study of the character "truncate wings" in *Sciara coprophila* (Metz 1927). Truncate is a recessive autosomal character, somewhat similar in appearance flies that have hatched. Although the culture method is by no means perfect as yet, it is adequate and reliable for present purposes.

3. *Temperature conditions*

Sciara is resistant to cold; the only effect of low temperature seems to be a retardation of the rate of development. The larvae are very sensitive to heat, however, and 29°C is lethal if maintained more than a short time. Higher temperatures are immediately lethal. In the laboratory the cultures are kept in an incubator with a temperature range of 22°–24°C. Moisture conditions are regulated by placing a large flat pan of water on the lowest shelf in front of an electric fan which is in continuous operation. Under these conditions the life cycle of *S. coprophila* occupies about a month, divided approximately as follows: egg stage 5–6 days; larva 14–15 days; pupa 3 4 days; adult 5–8 days. Twelve to fourteen successive generations may be grown in the course of a year.

4. *Breeding technique*

The type of inheritance found in Sciara necessitates certain variations from the usual breeding technique employed with other animals. *S. coprophila* is "monogenic," individual females typically giving "unisexual" progenies. (One bisexual line arose as a mutation (METZ 1931) and is being studied). Occasionally there will be one or more "exceptional" males in a female progeny, or "exceptional" females in a male progeny, in which case sib matings can be made, but usually such inbreeding is not possible and it cannot be relied on as a method of studying linkage. The precise methods employed will be given more fully in the section on linkage.

In maintaining mutant stocks in the laboratory it has not proved feasible to combine several characters in one stock as is done in Drosophila work. When this has been attempted, the stocks have lost viability, despite every care. For this reason pedigreed stocks of each line have been kept. In practically all the work pair matings are used in maintaining stocks, mutant females being out-crossed to wild type males from a wild stock every generation to keep the lines viable. Even with these precautions the mutant stocks frequently show poor viability. The wild stocks also show considerable variation in this respect. These fluctuations do not appear to be related to any immediate environmental effect, for it rarely happens that more than one stock is in poor condition at a given time, although all the flies are kept under the same conditions. Likewise fluctuations in viability are not usually associated with seasonal changes.

GENERAL ACCOUNT OF THE MUTANT CHARACTERS AND THEIR OCCURRENCE

It is exceedingly difficult to secure satisfactory mutant characters in Sciara. This is due in part to the remarkable resistance to radiation shown by these flies, and in part to the physical characteristics which conceal all except the most obvious changes. Furthermore, the type of inheritance in this species tends to conceal recessive characters because the progenies are essentially unisexual and consequently sib matings are rare. Finally, the selective segregation occurring in the male prevents the transmission of paternal characters through the male line.

The normal rate of appearance of mutant characters in nature is very low, as witnessed by the fact that in hundreds of cultures of *Sciara coprophila* derived from five different localities and cultivated in the laboratory for a number of years in both pair matings and mass matings, only three mutant characters were found prior to 1930. All of these were recessives; two were sex-linked (swollen and narrow) and one was autosomal (truncate). During the course of the present study, two more sex-linked recessive characters (miniature and round) and two more autosomal characters (Delta, a dominant; oval, a recessive) have arisen spontaneously. Other mutant characters may have been spontaneous in origin, but since they came from lines subjected to X-ray treatments this cannot be concluded with certainty.

In an effort to increase the mutation rate, adult flies of both sexes were X-rayed. The treatments were given at the Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, New York, and at the Marine Biological Laboratory, Woods Hole, Massachusetts. In all cases a tungsten target was used and a 1 mm aluminum filter for intercepting the soft rays. In the work done at Cold Spring Harbor the dosage was determined by means of a Victoreen Dosimeter, while at Woods Hole mathematical calculation was employed.

Sciara is able to withstand very high dosages of X-rays as compared with most other organisms, and shows no effect from dosages commonly employed in Drosophila work (1,000 to 5,000 *r* units). The range of treatments found to be effective was from 7,000 to 20,000 *r* units. It is not certain that the X-ray treatments actually induced the mutations. However, mutations did occur in cultures that had been treated.

It is usually assumed that it is not profitable to work with dosages which produce greater than 50 percent sterility, but in a form in which mutation is rare and which is relatively resistant to artificial means of producing mutant characters, it is feasible to use dosages which cause complete sterility in as many as 70 percent of the cases. The percentage of sterility produced is extremely variable from time to time, even when the X-ray treatment is essentially the same and the flies are similar.

Although some of the mutant characters came from treated cultures, the genetic data make it seem probable that these as well as the other mutations represent actual gene changes rather than chromosome abnormalities. The origin and history of each will be taken up in chronological order.

Each new mutant character was tested with respect to constancy, selective segregation and linkage. All the new characters described here are dominant wing peculiarities. One was found by Mrs. C. S. MAURICE, the remaining five by the writer. Their characteristics may be readily observed by examination of the photographs and comparison with the wild type wing shown in figure 3a.

In describing variations from the wild type, the terminology of wing venation used by JOHANNSEN (1909) is followed (fig. 2).

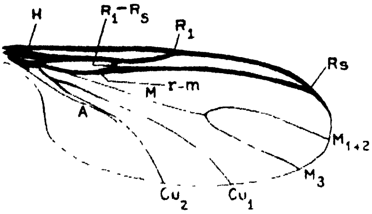

FIGURE 2 Diagram after JOHANNSEN, showing Sciara wing venation.

H = humeral cross-vein at base of wing
R = radius
R_1 = first branch of radius
R_s = posterior branch of radius
M = media
M_{1+2} = anterior branch of media
M_3 = posterior branch of media
Cu = cubitus
Cu_1 = anterior branch of cubitus
Cu_2 = posterior branch of cubitus
A = anal vein
r—m = radio-median cross vein
$R_1 - R_s$ = cross branch of radius.

1. *Curly*

a. Origin. Curly is an autosomal dominant, found April 15, 1930 in a single F_2 female from X-rayed + male. Since the character is a dominant, it is probable that it would have appeared earlier had it been the result of the X-ray treatments.

b. Description. The wing appears to have expanded normally and then become curled forward from the posterior end of the wing toward the head (fig. 3b). It is extremely variable, ranging from a barely perceptible bend in the wing to an extremely tight curl. There is no irregularity of venation or unusual pigmentation. If the curl is very extreme, the wing appears to be distorted and can be flattened out only with effort. This sometimes makes classification difficult when crosses are made involving other wing characters.

c. Occurrence. Not only is Curly variable in appearance, but it is inconstant as well; it does not always show when the gene is present. In a series of tests crossing virgin females heterozygous for Curly to wild type males from stock, the progeny from 322 pair matings were: 17,371 wild type and 8,161 Curly. That is to say, of 25,532 offspring 68 percent were wild-type

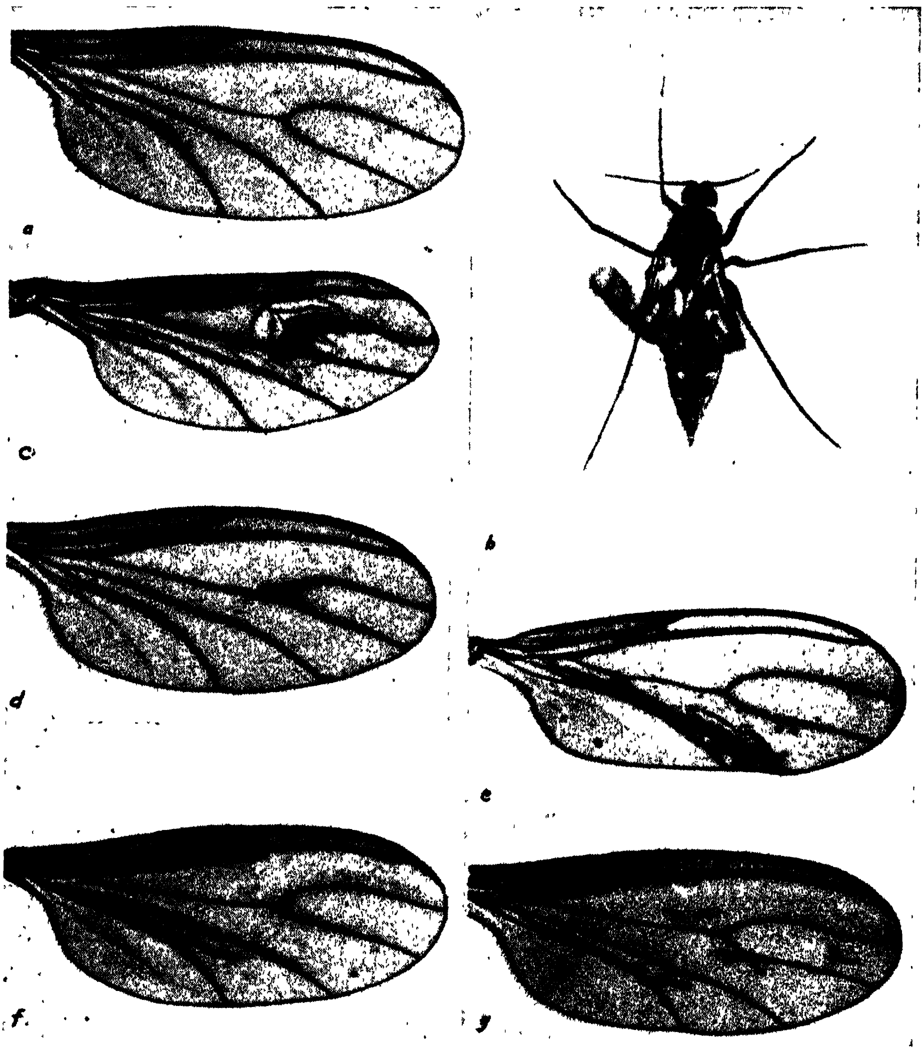

FIGURE 3. Photographs of Sciara showing:

(a) normal or wild-type wing
(b) Curly fly, whole mount
(c) Blister wing
(d) Delta wing
(e) Fused wing
(f) Dash wing
(g) Varied wing

All photographs ×35 except (b) which is ×5 and 32 percent were Curly. This deviates widely from the 1:1 ratio which would be expected if the character were constant and of normal viability.

In a test involving 467 flies (the total progeny of four pair matings tested in another connection) Curly was found to be concealed when actually present in 17.7 percent of the flies. This fact must be taken into account in analyzing linkage values.

d. Genetic behavior of the males. Since Curly is inconstant, the transmission of maternal characters through the male is correspondingly obscured. From 18 tests in which a wild type female from stock was mated to a Curly male (the son of a Curly mother), 709 wild type and 519 Curly offspring arose, whereas, all the flies would have been Curly if the character were constant. Curly was concealed in more than 57 percent of the offspring. In four of these eighteen cases, the progeny were tested further: of the 80 apparently wild-type flies (save 13 which were infertile) each gave some Curly offspring, showing that all the wild type flies were genetically Curly.

From 29 tests of Curly males which had inherited the character from their fathers, all of the offspring were wild type except three questionable flies. One of these appeared to be Curly, but on testing was found to be wild type; the other two had rumpled wings and may possibly have been Curly, but were not tested. In stock cultures flies appear occasionally which have rumpled wings but which on breeding prove to be wild type, and since this wing characteristic is transitory, it is probably an environmental effect. It is probable that the two flies not tested belong to this class. In any case the usual genetic behavior of the Curly male is typical.

2. *Blister*

a. Origin. Blister is a dominant autosomal character which appeared first May 12, 1930 in a single F_2 female from X-rayed wild type grand parents. If the character arose as the result of the X-ray treatments it must have been due to some latent effect, since the character, although a dominant, did not appear until the second generation after treatment.

b. Description. Blister appears as a large bubble in the general area of the posterior branching of the media vein (fig. 3c). In figure 2 this area is represented as the juncture of the media vein with M_{1+2} and M_3. It looks as if the wing had failed to expand normally and that an excess of liquid had collected to form this blister-like structure. The wings of any one fly are approximately symmetrical, although the size of the blister is somewhat variable. Often the whole wing is much shrunken and distorted. There is no abnormality of pigmentation and no irregularity of venation. The size of the blister varies greatly in different flies. In the extreme form the whole middle portion of the wing is involved and is pulled out of shape, but in other cases the blister may be so slight that it produces only a swelling of the media vein at the point of origin of the anterior and posterior branches. In this form it is very similar to the mutation Delta.

c. Occurrence. Blister flies are of good viability and give large progenies. 29 heterozygous Blister virgin females crossed individually to + males from stock gave 1,013 + and 846 Blister. Fifteen additional matings between heterozygous Blister females and their own Blister brothers gave 486 + and 411 Blister flies. Summarizing the results: 44 heterozygous females gave 1,499 + and 1,257 Blister flies, or a proportion of about 54 percent + to 45 percent Blister. The deficiency of the Blister class probably indicates a lower viability of the mutant flies, for on the whole the average is not far from the expected 1:1 ratio.

Blister is constant and is manifest when present, as shown by the ratios and by tests of 31 wild type sisters of Blister. The latter produced a total of 4,547 flies, of which only three were noted as questionable. One of these was tested and found to be wild type; the other two were not tested.

d. Genetic behavior of the males. Blister males derived from Blister mothers transmit the character as if they themselves were homozygous for the mutant gene. In 18 crosses of wild type virgin females by Blister males of the above type, there were 1,548 offspring all Blister.

On the other hand, Blister males which inherit the character from their fathers do not transmit it. Nineteen such males were tested by crossing to wild-type virgin females from stock. The total offspring were 1,525 wild type, and 1 questionable fly which was infertile.

3. *Delta*

a. Origin. Delta is an autosomal dominant character which was found June 6, 1930. It appeared simultaneously in several flies of a mass culture from pure stock of the "bisexual" line and had no history of X-ray treatment.

b. Description. Distinguishing features are relatively constant (fig. 3d). Of these, the more obvious one is the swelling of the juncture of the media vein with its anterior and posterior branches (fig. 2). The other feature is the thickening of the marginal ends of the veins termed Cu_1, Cu_2, M_{1+2}, and M_3. Sometimes the small cross-vein $(R_1 - R_s)$ is thickened noticeably. Delta is quite variable in appearance, ranging from a barely perceptible swelling of the regions designated above to a distorted, blistered wing, indistinguishable from the character Blister.

c. Occurrence. From 25 tests of heterozygous Delta females crossed to wild type males from stock, the total offspring were 1,283 + and 1,172 Delta flies. In addition, from 26 heterozygous Delta females crossed to wild type or Delta brothers which would be expected to breed pure for wild type the offspring were 1,153 wild-type and 954 Delta. Summarizing: 51 matings of heterozygous Delta females gave a total of 2,436+and 2,126 Delta flies, or approximately 53 percent+ to 46 percent Delta. The mutant class showed poorer viability than the wild type.

On the whole the character is constant, although occasionally a fly that appears to be wild type is found to carry Delta. Eighteen wild type sisters of Delta females were tested in this connection. Of these, one gave a progeny of 167 wild type flies and one questionable fly which was dead when found. Another female gave a progeny of 142 wild type and 9 questionable flies, which when tested further were found to be actually Delta. The remaining 16 females gave only wild type offspring.

d. Genetic behavior of the males. Transmission of the character through the male line is typical of the usual inheritance found in Sciara; males transmit to their offspring only the genes derived from their mothers. From 10 Delta males crossed to wild type virgin females from stock, Delta being maternal in origin, only Delta offspring arose. Nine Delta males were tested which had inherited the character from their fathers; all gave only wild type offspring.

4. *Fused*

a. Origin. Fused is a dominant autosomal character which first appeared in a single female September 8, 1931. This fly was the daughter of a female which had received an X-ray treatment of 20,000 *r* units.

b. Description. The most distinctive feature of this character is the irregular fusion of the anterior and posterior branches of the cubitus vein (fig. 3e). This is always present, although the amount of fusion varies. The media vein is slightly irregular and appears to be the result of a puckering of the wing toward the lower margin, occasioned by the fusion of veins in that area. There may be a general increase in the amount of pigmentation of the whole wing, especially in the region anterior to the posterior branch of the radius vein (R_s) but this is not always present. The cross-vein ($R_1 - R_s$) may be markedly thickened. In a given fly, the wings are essentially symmetrical.

c. Occurrence. Fused flies are prolific and the character is constant. Heterozygous females, when crossed to wild type males from stock give offspring that closely approximate a 1:1 ratio. Twenty-two pair matings of this type gave 1,327+and 1,410 Fused flies, a ratio of approximately 48+ to 51 Fused. The fact that the mutant class is larger than the wild type shows the excellent viability of the mutant flies.

To test the constancy of the character, nineteen virgin wild type sisters of Fused females were crossed to wild type males from stock. These gave only wild type offspring.

An additional test was made by crossing ten wild type sisters of Fused which were not certainly virgin (they might have mated with their wild type brother) by wild type males from stock. Nine of these gave only wild type offspring. The tenth gave only Fused offspring: 1 female and 88 males. To determine the source of Fused in these males, some were tested further; they were found to breed true for wild type. Since it is known that the males transmit only the characters derived from their mothers and these males gave only wild type offspring it is to be concluded that Fused was derived from the father, and that it was the father rather than the mother which was genetically Fused while appearing to be wild type. Apparently Fused is not often concealed for in the tests thus far made this is the only exceptional case.

d. Genetic behavior of the males. The genetic results of tests of Fused males are similar to the results obtained with other mutant characters. From 37 cases in which a wild type female from stock was crossed to a Fused male which had derived Fused from his mother, the offspring were Fused without a single exception. Likewise in 36 counts in which a wild type female was crossed to a Fused male that had received the character from his father, all of the offspring were wild type. Again the males are shown to breed as if homozygous for characters of maternal origin.

5. *Dash*

a. Origin. Dash is an autosomal dominant character which first appeared October 3, 1931. The first fly was a male, the son of a female which had been treated with 20,000 *r* units of X-rays. This male was mated successively to four different females and all the offspring showed the character. In view of further tests with this character and its transmission through the male, there can be no doubt that the mutant gene was derived from the female parent.

b. Description. The most obvious and constant characteristic is the presence of a very pronounced structure between the anterior and posterior branches of the cubitus vein (fig. 3f). This resembles the usual wing veins in color and in sharpness of outline. Its direction of slope is downward from the posterior branch of the cubitus vein toward the anterior branch. The spot is elongate and slender, variable in size, and sometimes attached at one end to the posterior branch of the cubitus vein, in which case it gives the appearance of an extra vein. More often it exists as a separate structure lying between the two veins.

Occasionally, the character is more extreme for several generations, due perhaps to the presence of undetected modifiers. This variation is characterized by the presence of numerous scattered blobs of pigment, suggesting the character Varied. Despite the altered appearance, the spot between the two branches of the cubitus vein remains distinct. Usually the Dash wing has a cloudy appearance, with a greater amount of pigmentation in the anterior region near the wing margin.

c. Occurrence. Dash affords no exception to the usual type of inheritance found in Sciara. The character is constant and the flies are viable. 23 crosses of heterozygous Dash females by wild type males from stock, gave 1,131 + and 1,150 Dash. Seven crosses of heterozygous Dash females by wild type brothers (which would breed pure for wild type) gave a total of 282 + and 291 Dash offspring. Summarizing: 30 heterozygous females gave 1,413 wild type and 1,441 Dash flies, about 49 percent of the total being wild type and 51 percent Dash—approximately a 1:1 ratio.

d. Genetic behavior of the males. Transmission of the character through the male differs in no way from that observed previously in Sciara. Twelve Dash males which had received the character from their mothers were tested; their offspring without a single exception were Dash. Fourteen Dash males which had received the character from their fathers were tested; their offspring were all wild type.

6. *Varied*

a. Origin. Varied is a dominant, autosomal character which appeared first October 14, 1931 in one male, the son of a female which had been subjected to 13,000 *r* units of X-rays. This male was bred to several females and transmitted the character to all of his daughters and all but three of his sons. In view of the usual genetic behavior of Sciara males, the fact that practically all the offspring received the character (the three exceptional flies probably being Varied since the character has been shown to be inconstant), indicates that the mutant gene came originally from the treated female.

b. Description. This character is extremely variable in appearance. Figure 3g shows a characteristic wing. The chief feature is a general duskiness of color accompanied by scattered blobs of material resembling vein fragments. These spots are variable in size, shape, number and location. There may be several such fragments, well-distributed over the wing surface, or there may be only one, in which case it is usually confined to the region between the anterior and posterior branches of the cubitus vein. Often there is an intermittent fusion of the posterior and the first branches of the radius vein, but there is no regularity about this, either as to the amount or place of fusion. In its most simple manifestation the character looks very much like Dash, described previously. The two wings of a given fly are seldom symmetrical, although both wings usually show the same degree of modification.

c. Occurrence. Flies showing the character Varied emerge late in any culture, after the majority of the wild type flies have hatched. The division of the hatching period into two phases is especially striking in female progenies derived from heterozygous mothers. If the number of offspring is small, it frequently happens that only the wild type flies hatch. Eighteen heterozygous Varied females crossed to wild type males from stock gave 1,082+and 521 (or 32 percent) Varied. Eleven additional progenies were tested and the offspring which appeared to be wild type were tested further to determine their genetic constitution. The initial count was: 17 wild type (which were dead when found); 681 wild type (which were tested); 255 Varied. After testing the final result was found to be: 543 wild type and 410 Varied. The 17 dead flies and the flies which gave no offspring were classed as wild type in the final count, weighting the results in the direction of that class. Even with this possibility of error, 43 percent of the total offspring were found to be Varied. Of the 410 Varied flies in the final count, 155 (37 percent) had appeared to be wild type before being tested.

d. Genetic behavior of the males. As in the cases previously described, the males transmit only the genes of maternal origin, although Varied does not always show when the gene is present. From 25 pair matings (wild type virgin females crossed to Varied males from Varied mothers), the progeny were 151 wild type and 2,377 Varied. Although most of the offspring showed the Varied character as expected, about 6 percent appeared to be wild type. The latter were probably genetically Varied.

In testing the transmission of Varied through males which had derived the character from their fathers, 20 pair matings were made. Eighteen of these gave only wild type offspring as expected, but the remaining two showed unusual behavior. One gave only wild type offspring save for three females which appeared to be Varied (not tested). The second gave only wild type offspring save for one male which was Varied and which was crossed successively to 5 wild type virgin females from wild stock. One female gave no offspring. Three females gave female progenies in which every fly was Varied, indicating that the Varied male transmitted only the character of paternal origin. The remaining female gave 44 Varied females and 1 wild type male. (This male was tested but gave no offspring.) Many of the Varied females from these four progenies were tested; they gave progenies typical of the usual heterozygous females. Although a stock derived from this source was kept in the laboratory for almost two years, neither females nor males showed any further tendency to breed differently from flies of the regular Varied stock.

The appearance of these exceptional flies at once suggests contamination, a possibility which cannot be ruled out completely. However, if contamination had occurred through the entrance of a Varied male (Varied of maternal origin) into the cultures, as the results suggest, a larger number of Varied flies should have appeared. Each progeny consisted of more than 100 flies, yet in the first exceptional case only three unusual flies arose, and in the second case only one.

The fact that Varied is inconstant, particularly in male progenies, further complicates the situation, but as they stand, the results suggest that this exceptional case may have come about as the result of a reverse segregation of chromosomes at the first spermatocyte division, which resulted in the retention of the chromosome of paternal origin, rather than the chromosome of maternal origin. If so, the male in this instance showed reversal of the usual type of inheritance found in Sciara.

LINKAGE

The primary purpose of the linkage tests is to identify the different chromosomes and to ascertain whether or not they all exhibit the same type of segregation. Unfortunately the type of inheritance involved here presents certain difficulties for the study of linkage because it is necessary in each case to back-cross individuals of the sex in which crossing over occurs. In spite of this difficulty, it is believed that the evidence warrants the tentative conclusion that all three autosomes have been identified.

When the present study was begun, two linkage groups were already known, one representing the sex chromsomes and the other representing the first pair of autosomes, the latter identified by the character truncate. Since none of the new characters was sex-linked, it was obvious that they did not belong to Group I.

Since backcrosses of heterozygous males are unsuitable for linkage tests because of selective segregation of chromosomes occurring during spermatogenesis, such tests had to be made in the manner indicated by the following examples. In testing the recessive character truncate with a dominant such as Blister, truncate females were crossed to Blister males (from Blister mothers). The hererozygous daughters were mated to truncate males from pure stock and the progeny counted. In testing two dominants together such as Dash and Blister, Dash females were mated to Blister males (from Blister mothers); the heterozygous daughters were outcrossed to wild type males from wild stock and the progeny were counted. Reciprocal crosses were made in both types of tests.

The results of the linkage experiments are summarized in table 1. Curly proved to be the only character representing the second pair of autosomes (Group III) and had thus to be used in spite of its inconstancy.

1. *C*×*tr*. Curly was first crossed with the recessive truncate. The two characters did not appear to be linked, but the results were obscured by the inconstancy of Curly. Since the required data were important, the tests were repeated and all the questionable flies were tested further. This entailed the testing of all wild type flies for Curly, all truncate flies for

Curly, extreme Curly flies for truncate, and shrivelled flies for both truncate and Curly. A small number of weak flies were dead soon after hatching and could not be tested further. Some of the flies that were tested proved to be infertile. In the final analysis the flies of these two groups were included in whatever class they appeared to belong.

There is no doubt some error in the final calculations due to the fact that the classification of the infertile flies, of necessity, followed the original classification given them. This classification was probably correct in many cases, but the extent of accuracy cannot be known. There were in this group 111 normal, 105 truncate, 9 Curly, and 1 fly classed as wild type which may have been truncate, (it was classed as shrivelled at first but was tested only for Curly). If some of these infertile wild type flies were actually Curly, the Curly class would be larger at the expense of the wild

TABLE 1

	CASES	TOTAL FLIES	PERCENTAGE CLASSIFICATION			
			+	tr	C	Ctr
1. C×tr	15	2,009	25	23	29	22
			+	B	C	BC
2. C×B	35	1,855	37	25	18	17
			+	tr	B	Btr
3. B×tr	44	3,666	29	20	43	6
			+	Δ	C	ΔC
4. C×Δ	29	2,727	29	28	18	24
			+	tr	Δ	Δtr
5. Δ×tr	35	3,985	25	25	26	22
			+	B	F	BF
6. B×F	30	2,345	3	46	46	4
			+	Δ	F	FΔ
7. F×Δ	13	650	6	35	56	9
			+	tr	F	Ftr
8. F×tr	11	565	28	20	31	20
			+	C	F	FC
9. F×C	8	361	32	15	34	17
			+	B	D	BD
10. D×B	16	890	29	41	21	7
			+	Δ	D	ΔD
11. D×Δ	10	436	30	25	22	22
			+	tr	D	Dtr
12. D×tr	15	701	2	34	62	.8
			+	C	D	DC
13 D×C	13	766	20	23	31	24
			+	tr	V	Vtr
14. V×tr	43	2,847	.6	57	40	.6

+ indicates wild type.

Detailed data from which this table was prepared have been placed on file with "Genetics" and may be consulted by anyone interested.

type class; and likewise, if some of the infertile truncate flies were Curly, the Curly-truncate class would be larger, with a corresponding diminution of the truncate class. The few infertile Curly flies might have been either Curly or Curly-truncate. There were 226 of these questionable flies which comprised 11 percent of the total offspring.

The offspring from 15 pair matings were tested with the results shown in table 1 (1). The total number of flies involved was 2,009, divided approximately as follows: 25 percent wild type, 23 percent truncate, 29 percent Curly, and 22 percent Curly-truncate. The conclusion seems warranted that Curly and truncate are not linked since each pair mating showed a clear division of the progeny into four approximately equal classes, even in the tests which had the greatest number of infertile flies. Since truncate was previously assigned to the first pair of autosomes, on the basis of earlier evidence, Curly can now be regarded as located in the second pair of autosomes (Group III), pending further analysis.

2. *C*×*B*. Turning to table 1 (2), the counts of 35 tests of Curly by Blister gave 1,855 flies of which 37 percent were wild type, 25 percent Blister, 18 percent Curly, and 17 percent Blister-Curly. Since Curly is inconstant, it is evident that a number of flies classed as wild type must have been genetically Curly, and likewise a number classed as Blister were genetically Blister-Curly. This accounts, at least in part, for the size of the wild type and Blister classes as compared with the Curly classes. The concentration of the averages into the wild type and Blister classes does not suggest that the characters are linked, for if they were, the Blister and Curly classes would be expected to be the largest and the wild type and Blister-Curly classes the smallest. This interpretation receives further support from the evidence presented in the following paragraph.

3. *B*×*tr*. Table 1 (3) shows the results of 44 tests of truncate by Blister, involving 3,666 flies. Thirty-four of these were shrivelled; the remainder were approximately 29 percent wild type, 20 percent truncate, 43 percent Blister, and 6 percent Blister-truncate. The most interesting feature here is the excessive number of Blister flies. The probable explanation is that extreme Blister causes a great distortion of the wing so that the whole shape is askew; truncate affects only the shape of the wing; when the two characters are present in the same wing, the presence of Blister so alters the shape of the wing that truncate is concealed. Furthermore, it is the Blister-truncate class which is deficient throughout and the Blister class which is in excess. If some of the flies classed as Blister were actually Blister-truncate, these two groups would tend to become more nearly equal. If the Blister-truncate group represented a crossover class, it would be expected that the wild type class also would be small, which is not the case. Blister is not linked to either truncate or Curly, so tenta-

tively at least, it can be stated that Blister is in the third pair of autosomes (Group IV). Thus each pair of autosomes is represented by a character, and it remains to be shown in which linkage groups the remaining four mutant characters belong.

4. *C*×Δ. Delta was studied first in its relation to Curly with the results set forth in table 1 (4). From 29 pair matings 2,727 offspring arose. Twenty nine percent were wild type, 28 percent Delta, 18 percent Curly, and 24 percent Delta-Curly. Here the Curly and Delta-Curly classes are smaller than the wild type and Delta classes. Again it must be emphasized that the inconstancy of Curly obscures the true results. If the Curly gene were present in some of the wild type and some of the Delta flies, as was probably the case, the Curly and Delta-Curly classes would be proportionately increased. This would tend to establish the 1:1:1:1 ratio expected in characters that are not linked.

5. Δ×*tr*. Delta was next tested with truncate, as shown in table 1 (5). The 3,985 offspring from 35 pair matings were approxmately as follows: 25 percent wild type, 25 percent truncate, 26 percent Delta, and 22 percent Delta-truncate. Twelve shrivelled flies were not included in the above averages. The flies showing both mutant characters evidently have a lower viability than those of the other classes, for on the average, the Delta-truncate class is the smallest. This is probably not due to any difficulty in classification, for both characters are definite and easily recognized, even when present in the same wing. Truncate and Delta are relatively constant; the percentage of cases in which the characters are concealed is too small to explain the deficiency of the Delta-truncate class on that basis. Furthermore, the three other classes, (wild type, truncate, and Delta) are very nearly equal in size and show no undue concentration of flies in any one class. In three of the matings the progeny were divided into classes almost equal numerically. Evidently Delta and truncate are not linked.

6. Δ×*B*. Having found that Delta was not linked either to Curly (Group III) or truncate (Group II) the inference was that it must be linked to Blister (Group IV). The proof of this by crossing Blister with Delta was difficult, chiefly because the two characters are so similar that a double mutant class, if it did arise, would never be distinguishable as such. In addition, the extreme Delta wing so closely resembles Blister that a classification of these groups could never be accurate. A test to secure the information indirectly was made in the following manner. Blister virgin females were crossed to Delta males, the sons of a Delta female. (The reciprocal test was also made.) From individual female progenies arising thus, 26 of the most extreme flies, which might be both Blister and Delta, were outcrossed to wild type males from stock. If any of the females were Blister-Delta, this should be apparent in the progenies. Without exception,

however, there were only two types of offspring from each mating; these were either wild type and Blister or wild type and Delta; never were there three classes. Such results suggest that the two characters can be combined in one fly only with difficulty if at all, and that perhaps they are allelomorphs. This impression is further strengthened by the fact that it is difficult, if not impossible to get either of these characters in a homozygous condition.

7. *B*×*F*. Fortunately the linkage of Blister and Delta can be shown through a study of their respective relationship to another character, Fused. The results of crossing Blister with Fused are shown in table 1 (6) The progeny fall into two major classes, Blister and Fused, in almost equal numbers. Of the total offspring from 30 pair matings, 46 percent are Blister and 46 percent Fused. The cross-over classes are exceedingly small, averaging approximately 3 percent wild type and 4 percent Blister-Fused. Not only is the linkage apparent from the averages of all cases, but the same type of result is characteristic of each mating throughout the whole series. In no case is there a grouping of the progeny into four approximately equal classes.

8. Δ×*F*. The results of crossing Delta with Fused are similar to those obtained from crossing Blister with Fused. Although the number of tests is less and the averages are smaller, the grouping of the progeny into two major classes is definite, whether viewed from the standpoint of individual matings or of the combined averages. The results are given in table 1(7). From 13 pair matings the progeny are divided as follows: 6 percent wild type, 35 percent Delta, 56 percent Fused, and .9 percent Fused-Delta. In some individual matings the Fused and Delta classes are about equal numerically, but on the whole the Fused class is considerably larger. This probably indicates a poorer viability of Delta.

9. *F*×*tr*. That Fused appears to be linked to both Blister and Delta is further supported by the fact that it appears to be independent of both truncate and Curly. The results of the Fused-truncate crosses are given in table 1 (8), the averages for 11 pair matings being 28 percent wild type, 20 percent truncate, 31 percent Fused, and 20 percent Fused-truncate (5 shrivelled flies omitted). In some of these matings the truncate and truncate-Fused classes are smaller than the wild type and Fused classes, which in turn affects the final averages for these groups. Since the results are not typical of linkage (if the characters were linked the truncate and Fused classes would be expected to predominate), it is probable that the poor viability of truncate is responsible. In three of the eleven pair matings, the progeny were divided into four classes of almost equal size.

10. *F*×*C*. The final counts of crossing Fused with Curly (table 1 section 9) indicate, when the inconstancy of Curly is considered, that the two

characters are not linked. From 8 pair matings the count was 32 percent wild-type, 15 percent Curly, 34 percent Fused, and 17 percent Fused-Curly. Doubtless some of the wild type flies were genetically Curly and some of the Fused flies were actually Fused-Curly. In summary it may be said that Blister, Delta, and Fused have been shown to be linked and to represent the third pair of autosomes (Group IV).

11. *D*×*B*. Dash was crossed to Blister and to Delta with results essentially alike in both cases, again confirming the linkage of Blister and Delta. In Table 1(10) the averages of 16 crosses of Dash and Blister are: 29 percent wild type, 41 percent Blister, 21 percent Dash, and 7 percent Blister-Dash. The Dash class is much smaller than either the wild type or Blister classes, due probably to poor viability. The small size of the double mutant class would suggest linkage were it not for the fact that the wild type class (which would be the other cross-over class if this were the case) is so large. Probably the flies having two mutant genes were extremely weak and many failed to hatch.

12. *D*×Δ. The results of crossing Delta with Dash are given in table 1(11). Again the two Dash classes are smaller than the wild type and Delta classes but the differences arenot as large as inthe Dash and Blister crosses. The progeny from 10 pair matings are: 30 percent wild type, 25 percent Delta, 22 percent Dash, and 22 percent Delta-Dash.

Dash could not be tested with Fused because of the similarity of the two characters.

13. *D*×*tr*. The characters Dash and truncate were found to be linked. Table 1(12) shows that there are only two main classes of progeny, truncate and Dash in the backcrosses. The 701 offspring from 15 pair matings are: 34 percent truncate, 62 percent Dash, with the crossover classes represented by only 2 percent wild type and .8 percent Dash-truncate. Two shrivelled flies are not included. On the whole, truncate seems to be less viable than Dash, for there are almost twice as many Dash as truncate flies. The truncate class is deficient in the matings where the total number of progeny is small. In five of the fifteen matings the progenies are fairly large; in these, truncate and Dash are present in about equal numbers. The crossover classes are almost non-existent.

14. *D*×*C*. On the basis of the tests described above (11, 12, and 13) Dash was thought to be located in chromosome Group II. To verify this conclusion it was tested with Curly. It was anticipated that the two characters would be found to be independent of each other, but since a study of the relationship between Dash and Curly would provide additional information concerning the relationship of truncate and Curly (truncate being linked with Dash), the analysis was undertaken in some detail.

The results of the first tests of Dash and Curly are as follows: 13 cases gave 1 percent shrivelled, 33 percent wild type, 23 percent Curly, 30 percent Dash, and 10 percent Dash-Curly. Since the inconstancy of Curly proved such an obstacle to interpretation of these data, a second test was made in which the non-Curly flies from 5 pair matings were tested further. The initial count was 7 percent shrivelled, 39 percent wild type, 24 percent Curly, 18 percent Dash, and 10 percent Dash-Curly. After testing, however the percentages became: 1 percent shrivelled, 24 percent wild type, 24 percent Curly, 6 percent Dash, and 43 percent Dash-Curly. The infertile flies and the dead flies which could not be tested were tabulated in whatever class they appeared to belong, thus unavoidably introducing some degree of error. This group comprised 13 percent of the total offspring. In the revised count there was an unusually large number of Dash-Curly flies, due probably to the presence of unidentified modifiers which affected Dash to some extent, causing many Dash wings to resemble also the least extreme form of Curly. Since this type of variation frequently occurs and since it was present in pure Dash stock at the time these experiments were conducted, this explanation seems plausible.

The results of the second test were analyzed further, omitting all doubtful flies, and considering only the flies of which the true classification was known. Of 171 wild type flies tested, 42 percent proved to be genetically Curly. Of the 122 Curly flies, 62 percent proved to be Dash-Curly. Correcting the results of the first Dash×Curly experiment on the basis of these percentages, (transferring 42 percent of the wild type class to the Curly class and 62 percent of the Curly class to the Dash-Curly class) the final averages become: 20 percent wild type, 23 percent Curly, 31 percent Dash and 24 percent Dash-Curly. The "corrected" averages are shown table 1(13).

Although there is some inaccuracy in drawing conclusions from such indirect analysis, it seems clear that Curly and Dash are not linked, as the wild type and Dash-Curly flies are too numerous to be considered as crossover classes. These data also further support the conclusion that Curly is not linked to any of the other mutant characters and hence represents the only gene thus far identified in the third pair of chromosomes (III).

15. *V*×*tr*. It is impossible to study the linkage of Varied with any character save the recessive truncate, because Varied has certain features in common with all the others and accurate classification of the progenies would be impossible. Fortunately the tests of Varied with truncate provide all the data needed. As mentioned previously, Varied is not constant; some flies in Varied cultures appear to be wild type when they are genetically Varied. For this reason, all the flies classed as wild type in the Varied-truncate crosses were tested further to verify their classification. In these tests, Varied hatched late in every mating; practically all the truncate

flies hatched before Varied flies began to hatch. This is typical of the Varied cultures in general.

As shown in table 1(14), 43 pair matings were studied. In 23 cases there were no wild type flies to be tested. Of the 53 wild type flies tested more than half were found to be Varied. In the final calculation, among 19 wild type flies, 5 were known to be wild type, 2 were probably wild type but possibly Varied, and the remaining 12 were infertile but were classed as wild type because of their appearance. This whole group formed less than .6 percent of the total progeny. The Varied-truncate group also was exceedingly small, the total number of flies being 18, (less than .6 percent of the total progeny). The flies were mainly of two types, truncate and Varied, the averages being 57 percent truncate and 40 percent Varied. (Only the final averages are shown in table 1). It seems evident from these results that Varied belongs to the truncate-Dash linkage group (II).

Among the tests of wild type females from this series of experiments, four of the matings gave unusual results. These wild type females, heterozygous for truncate, were outcrossed to wild type males from wild stock 2567. The progeny which were expected to include only wild type and Varied contained some truncate flies as well. Further analysis showed that the 2567 stock was contaminated with truncate; the appearance of truncate in these further tests with Varied-truncate was undoubtedly due to this factor rather than to any unusual behavior of the Varied and truncate lines.

SUMMARY

(1) Six new wing characters in *Sciara coprophila* have been studied, all of which are autosomal dominants.

(2) Each character has been shown to segregate selectively in the male so that only characters of maternal origin are transmitted.

(3) A study of linkage has shown that all (except the "limited") chromosomes have now been identified by means of mutant genes and that accordingly the autosomes may be designated as follows:

Chromosome II—truncate, Dash, Varied
Chromosome III—Curly
Chromosome IV—Blister, Delta, Fused

It is not known which pair of chromosomes corresponds to any particular linkage group cytologically, except in the case of the sex chromosome. Since all of the autosomes are paired in the male soma, it is evident that the unpaired chromosome is not an autosome but is the sex chromosome.

(4) The rate of crossing-over for the autosomes was found to be very small, which corresponds to the results obtained previously for the sex chromosomes (METZ and SCHMUCK 1931, METZ and SMITH 1931).

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PREFERENTIAL SEGREGATION IN TRIPLO-IV FEMALES OF DROSOPHILA MELANOGASTER

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INTRODUCTION

THE occurrence of non-random segregation in triplo-IV females of *Drosophila melanogaster* was reported in a preliminary note (STURTEVANT 1934) of which this is the full account. As there shown, and elaborated below, all the tested fourth chromosomes may be arranged in a series, of such a nature that each chromosome will (in a triplo-IV female) pass to the haplo-IV pole at meiosis more often than will any chromosome that lies above it in the seriation. Expressed in another way, if two chromosomes (A and B) are tested against any third chromosome (C), then C will "prefer" that one of the other two that lies higher in the series.

These relations are first described in the case where one of the IV chromosomes concerned carries genes from the X chromosome. The data on other triplo-IV females are then presented. An algebraic analysis that leads to quantitative predictions is then developed.

It should be remembered throughout that the relations discussed are between whole chromosomes, not individual loci—a circumstance made experimentally feasible by the fact that crossing over in this chromosome is negligible in frequency. The chromosomes concerned are designated by the names of the mutant genes they carry, but these genes are to be regarded only as convenient markers, making it possible to follow the behavior of the chromosomes concerned.

DUPLICATION EXPERIMENTS

Males from the stock of scute-10 (=achaete−2) were X-rayed and mated to yellow scute-D1 females. There resulted numerous wild type daughters, and two that showed some of the characteristics of scute. These proved to have two separate modifications of the scute locus added to the achaete of the rayed males; they are designated scute-10−1 and scute-10−2. The latter (scute-10−2) was found to be associated with a reciprocal translocation between the X and IV chromosomes. The break in X lies between the loci of silver and broad (to the right of the so-called "viability gene" of PATTERSON); that in IV lies between the known loci and the spindle attachment. Dr. J. SCHULTZ has examined the salivary glands of this

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translocation, and informs me that it is more complex than this, there being a second break in IV beyond the loci of the known genes (the piece of X being intercalated between the two ends of IV). The two new chromosomes (X with left end replaced by IV, left end of X on spindle-attachment of IV) may be obtained separately; the present study is based on flies carrying the shorter of the two as a duplication: that is, diploids plus left end of X on an extra IV spindle attachment. The "normal" X's of such hyperploids carried yellow in the experimental cultures, so that wild type body-color served as an index of the presence of the duplicating fragment.

When such flies (females) were made heterozygous for the "normal" IV chromosomes it was found (STURTEVANT 1934) that the duplication did not segregate at random with respect to these IVs. The two IVs themselves pass to opposite poles in 95 percent or more of the eggs (see below); but the duplication regularly shows a *preference* for one of them. To use the same example as previously cited, in the case of females of the constitution $\frac{y}{y}$ dupl $\frac{ey^{D}}{gvl}$ crossed with y (not ey^{D}) ♂, there resulted 673 $+^{y}\,ey^{D}$, 372 $+^{y}\,+^{ey}$, 354 $y\,ey^{D}$, 806 $y\,+^{ey}$; that is, the duplication passed

TABLE 1

Percentage of cases in which the scute-10-2 duplication went to the same pole as the IV chromosome entered in each column heading, when that chromosome was tested against the ones indicated in the first column. The values entered as probable errors are the probable deviations from 50.0 percent—i.e. .6745$\sqrt{1/4n}$. The significance of the various symbols, where these are not standard designations for mutant genes, are indicated in the text and in the heading of table 2

OPPOSED CHROMOSOME	PREFERRED CHROMOSOME								
	$+^{ci}\,ey^{R}$	sv	$ci\,ey^{R}$	ey^{D}	ci	$X\,ci$	sv^{na}	bt	ci^{D}
$+^{ci}\,ey^{R}$	50.0		48.5 ± 1.8		48.5 ± 1.4			41.4 ± 1.8	
sv		50.0	47.2 ± 1.3	47.8 ± 1.6	52.2 ± 2.5				43.9 ± 2.4
$ci\,ey^{R}$	51.5 ± 1.8	52.8 ± 1.3	50.0	49.1 ± 1.8	51.1 ± 1.0	47.4 ± 1.2	43.2 ± 0.7	40.4 ± 2.0	
ey^{D}		52.2 ± 1.6	50.9 ± 1.8	50.0			45.0 ± 1.2	39.2 ± 1.6	39.5 ± 1.5
ci	51.5 ± 1.4	47.8 ± 2.5	48.0 ± 1.0		50.0		46.9 ± 1.0		
$X\,ci$			52.6 ± 1.2			50.0	48.2 ± 1.3		
$+$, $y\,sc\,w^{a}$			58.7 ± 1.4	49.2 ± 1.9			47.5 ± 1.1		34.7 ± 1.9
sv^{na}			56.8 ± 0.7	55.0 ± 1.2	53.1 ± 1.0	51.8 ± 1.3	50.0		43.0 ± 1.4
+, San G			51.9 ± 1.3	61.1 ± 1.8			52.7 ± 2.2		46.3 ± 2.1
+, Bham			60.6 ± 1.6	52.6 ± 1.5			57.6 ± 1.7		51.3 ± 1.3
bt	58.6 ± 1.8		59.6 ± 2.0	60.8 ± 1.6				50.0	44.0 ± 1.2
ci^{D}		56.1 ± 2.4		60.5 ± 1.5			57.0 ± 1.4	56.0 ± 1.2	50.0
+, Tonto			63.0 ± 1.7	59.0 ± 1.7	53.9 ± 2.0				
bt^{D}			58.7 ± 1.3	61.7 ± 2.1			56.6 ± 1.7		50.4 ± 1.8
+, Red. M.			68.0 ± 3.1	60.4 ± 1.6			50.6 ± 1.9		51.5 ± 2.6
+. Arr. S.			61.6 ± 2.9	59.0 ± 2.0			63.1 ± 1.4		50.3 ± 1.9
+, Fla.			62.1 ± 1.9	58.4 ± 1.8					
ey		62.4 ± 2.7	59.2 ± 1.2				56.7 ± 1.3	43.7 ± 2.5	60.4 ± 2.7
+, Seattle			61.4 ± 1.8	62.9 ± 1.7			54.9 ± 1.3		
+, Sch. X			64.3 ± 1.3	61.9 ± 1.8					
$gvl\,ey^{R}$			63.8 ± 1.6	65.2 ± 1.4		62.5 ± 1.4	56.0 ± 1.7	55.0 ± 1.4	56.5 ± 1.7
ar	69.1 ± 1.5		70.0 ± 1.3	62.6 ± 2.2			58.0 ± 1.9		59.2 ± 1.8
gvl		65.2 ± 1.7	67.8 ± 0.9	67.0 ± 0.7	60.7 ± 2.6		57.8 ± 1.2	57.0 ± 1.3	55.3 ± 1.4
ey^{4}		66.4 ± 1.9	66.4 ± 2.2	64.0 ± 2.9			61.3 ± 3.5	57.2 ± 1.7	
M^{4}							69.4 ± 1.5	60.6 ± 2.7	
ey^{2}			64.5 ± 1.6	65.8 ± 2.4	65.8 ± 1.7		64.9 ± 1.5	65.8 ± 1.8	62.2 ± 2.5

TABLE 1 (*Continued*)

OPPOSED CHROMOSOME	PREFERRED CHROMOSOME								
	bt^D	*ey*	+, Sch. X	*gvl ey*^{R}	*ar*	*gvl*	*ey*^{4}	*M*^{4}	*ey*^{2}
+^{ci} *ey*^{R}					30.9±1.5				
sv		37.6±2.7				34.8±1.7	33.6±1.9		
ci ey^R	41.3±1.3	40.8±1.2	35.7±1.3	36.2±1.6	30.0±1.3	32.2±0.9	33.6±2.2		35.5±1.6
ey^D	38.3±2.1		38.1±1.8	34.8±1.4	37.4±2.2	33.0±0.7	36.0±2.0		34.2±2.4
ci						30.3±2.6			34.2±1.7
X *ci*				37.5±1.4					
+, *y sc w*^{a}				43.6±1.8		45.0±1.2			
sv^{na}	43.4±1.7	43.3±1.3		44.0±1.7	42.0±1.9	42.2±1.2	38.7±3.5	30.6±1.5	35.1±1.5
+, San G.		45.0±1.1		42.3±1.4		42.7±1.5			31.4±2.7
+, Bham		44.0±2.5		43.7±1.4		42.7±1.3			39.8±2.1
bt		56.3±2.5		45.0±1.4		43.0±1.3	42.8±1.7	30.4±2.7	34.2±1.8
ci^D	40.6±1.8	39.6±2.7		43.5±1.7	40.8±1.8	44.7±1.4			37.8±2.5
+, Toronto		49.4±2.1		47.2±1.7		42.7±1.5			
bt^D	50.0	52.1±1.7		43.2±1.5		39.0±2.1			
+, Red M		48.9±2.3		47.8±1.9		40.7±1.4			
+. Arr. S.		50.1±1.6		44.7±1.7		47.6±2.2			35.1±1.9
+, Fla.				42.9±1.3		50.2±1.3			
ey	47.9±1.7	50.0	47.5±1.5	44.9±1.1		44.7±1.1			
+, Seattle				51.1±2.1		50.6±2.1			
+, Sch X		52.5±1.5	50.0	53.1±1.4		41.4±4.4	50.0±3.7		
gvl ey^R	56.8±1.5	55.1±1.1	46.9±1.4	50.0	46.9±1.3	47.3±0.9	41.7±2.4		43.2±2.3
ar				53.1±1.3	50.0	40.5±1.1	44.7±1.8		46.5±1.7
gvl	61.0±2.1	55.3±1.1	58.6±4.4	52.7±0.9	50.5±1.1	50.0	50.8±1.1		51.3±1.1
ey^4			50.0±3.7	58.3±2.4	55.3±1.8	49.2±1.1	50.0	53.0±3.8	
M^4							47.0±3.8	50.0	47.8±3.5
ey^2				56.8±2.3	53.5±1.7	48.7±1.1		52.2±3.5	50.0

to the same pole as the *ey*^{D} chromosome in 67.0 percent of the cases. The result was the same whether the duplication was received from the same parent as *ey*^{D} (918/1398 = 65.7 percent) or from the same parent as *gvl* (561/807 = 69.5 percent), though in the latter case there resulted more than 50 percent recombination. The result was also the same in attached-X females carrying a Y chromosome (1118/1674 = 65.8 percent) and in separate-X females with no Y (361/531 = 67.9 percent).

TABLE 2

Preference ratios for scute-10-2 duplication in tests concerning a series of wild type IV chromosomes These came from a mutant stock (yellow^2 *scute*^{12} *apricot) and from the following wild stocks: San Gabriel Canyon, California; Birmingham, England; Tonto, Arizona, Redwood Meadow, California; Arroyo Seco, Pasadena, California, St. Augustine, Florida, Seattle, Washington*

OPPOSED CHROMOSOME	PREFERRED CHROMOSOME							
	+, *y sc w*^{a}	+, San G.	+, Bham	+, Tonto	+, Red M.	+, Arr. S.	+, Fla.	+, Seattle
ci ey^R	41.3±1.4	48.1±1.3	39.4±1.6	37.0±1.7	32.0±3.1	38.4±2.9	37.9±1.9	38.6±1.8
ey^D	50.8±1.9	38.9±1.8	47.4±1.5	41.0±1.7	39.6±1.6	41.0±2.0	41.6±1.8	37.1±1.7
ci				46.1±2.0				
sv^{na}	52.5±1.1	47.3±2.2	42.4±1.7		49.4±1.9	36.9±1.4		45.1±1.3
ci^D	65.3±1.9	53.7±2.1	48.7±1.3		48.5±2.6	49.7±1.9		
ey		55.0±1.1	56.0±2.5	50.6±2.1	51.1±2.3	49.9±1.6		
gvl ey^R	56.4±1.8	57.7±1.4	56.3±1.4	52.8±1.7	52.2±1.9	55.3±1.7	57.1±1.3	48.9±2.1
gvl	55.0±1.2	57.3±1.5	57.3±1.3	57.3±1.5	59.3±1.4	52.4±2.2	49.8±1.3	49.4±2.1
ey^2		68.6±2.7	60.2±2.1			64.9±1.9		

Tables 1 and 2 show the results of the tests of this kind that have been carried out. Examination of these tables will show that the 26 kinds of IV chromosomes that have been tested fall into a series, approximately in the order in which they are listed in the rows of table 1. The nature of this series is such that the duplication "prefers" any chromosome to those that lie below it in the series, and this preference is greater the further apart the two chromosomes concerned lie in the series. This is shown by the fact that the values entered in each column gradually increase from the top to the bottom of the column. There are irregularities in this increase, often great enough to be statistically significant; but the general trend is unmistakable. It is probable that the discrepancies, so far as they lie beyond those to be expected from errors of random sampling, are due to differential viability or (in a few cases) to uncertainties of classification for some of the IV chromosome characters concerned.[1]

The exact seriation shown in the tables is not to be taken as accurate in detail, though the general neighborhood in which each chromosome is placed is clearly correct. The method used in obtaining this sequence, together with a numerical scale for judging the relative degrees of difference concerned, will be described below.

It will be observed that there is no evident relation between the position of a chromosome in the seriation and the mutant genes it carries. The top and the bottom chromosomes are both characterized by carrying recessive eyeless mutants, as is also *ey*, that lies near the middle of the series. Seventh from the top is a wild type chromosome, seventh from the bottom is another; and between these lie several other wild-type chromosomes, at least some of which certainly differ from each other.

Several of the tested chromosomes are derived ones. The first, $+^{ci}ey^R$, (from Dr. C. B. BRIDGES), arose by reverse mutation from the third, *ci* ey^R. It is probable that these two are really identical in their preference properties. The *gvl* ey^R chromosome (also from Dr. BRIDGES) arose by crossing over between *ci* ey^R and *gvl;* it is intermediate, definitely different from both, and much more like *gvl*, from which its spindle-attachment is derived. The "+, Sch. X" chromosome was obtained (by Dr. J. SCHULTZ) by crossing over between ey^D and *ci*. Here the crossover is much lower in the seriation than either of the chromosomes from which it was derived. Its spindle-attachment comes from ey^D, which BRIDGES (1935) has shown to be a compound chromosome, with a diploid laterally attached duplication. This duplication may be important in determining the properties of the ey^D chromosome; it is not present in the "+, Sch. X" chromosome.

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[1] The position of ey^D is different from that reported earlier (STURTEVANT 1934) This is because one of the earlier experiments was carried out with another chromosome ("Scutenick"), not further studied here, that sometimes gives a slight eye defect. These data have been discarded.

The "X *ci*" chromosome arose as a crossover in one of my triplo-IV experiments. The *ci* (and the spindle-attachment) came from $ci\ ey^R$, the $+^{ey}$ either from ci^D or from *gvl*. In either case the crossover is intermediate but more like the chromosome from which its spindle-attachment came. Evidently there is no one locus responsible for the preference properties of a chromosome, but it is possible that the spindle-attachment end is most important.

The relation of the duplication to the IV chromosomes is evidently due to the fact that it contains IV chromosome materials. A similar (undescribed) duplication, found in the "shaven" stock, carries the loci from *y* to silver, inclusive, but presumably has an X spindle-attachment. Experiments with it, comparable to those in table 1, gave the following values (the numbers given represent percentage preference for the first-named chromosome, which is the higher one in the seriation): $sv-ci\ ey^R$, 54.0 ± 3.2; $sv-ey$, 46.6 ± 1.9; ci^D-ey, 48.7 ± 2.7; $sv-ci^D$, 47.5 ± 4.3. These values are clearly not significantly different from 50.0. Similar results were obtained with duplications 107 and 118 (DOBZHANSKY 1934), though here one of the IV chromosomes concerned was a wild type of unknown position in the series.

On the other hand, another duplication derived from an X-IV translocation (an undescribed mottled notch received from DEMEREC) gave clear indications of preference, although the fragment of X concerned is considerably longer than that of scute-10 – 2. The values obtained here were: $ci\ ey^R-+y\ sc\ w^a$, 60.0 ± 2.0; $+y\ sc\ w^a-gvl\ ey$, 57.4 ± 1.9; $+y\ sc\ w^a-gvl$, 66.9 ± 1.8. These are clearly significant deviations from randomness, in the same direction as the corresponding sc^{10-2} values.

TRIPLO-IV FEMALES

When three IV chromosomes are present in a single female, there are three possible types of segregation that give two chromosomes to one pole and one to the other (AB/C, AC/B, BC/A). With the existing material it has not been possible to determine the relative frequencies of all three types of segregation simultaneously in experiments in which contrary classes gave a viability control (except by the use of progeny tests, which have not been carried out). In some of the data the three frequencies were determined (without the viability control), while in others the viability control was obtained but only one of the three frequencies was determined.

Two samples of the raw data are shown in tables 3 and 4. In table 3 appear the results of testing a series of different chromosomes against ey^D and ci^D.[2] Here the type of segregation determined is ey^Dci^D/x (x indicates any other chromosome listed in table 3); the viability control is similar to that in a linkage backcross. Contrary classes should be equal; inspection of the table shows that in every case the ey^D character reduced the viability materially. Other experiments indicate that this is not due to the fact that the gene occurs most often in triplo-IV flies, for this condition evidently does not interfere with the viability appreciably—in some cases at least it appears to improve it slightly. The table shows a general tendency for the percentage of tested segregation type to increase as one passes from the top to the bottom of the series of table 1. As in that table, there

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[2] Both ey^D and ci^D are fully dominant when present in single dose in triplo-IV flies. It has not been found possible to distinguish such specimens from the corresponding diplo-IVs.

TABLE 3

Offspring of triplo-IV females of the constitution $ey^D/ci^D/x$, *mated to diplo-IV males not carrying* ey^D *or* ci^D.

x	ey^Dci^D	+	ey^D	ci^D	TOTAL	PERCENT ey^Dci^D/x
sv	181	242	250	347	1020	41.5
$ci\ ey^R$	67	82	163	168	480	31.1
ci	41	57	97	128	323	30.3
+, $y\ sc\ w^a$	44	36	49	97	226	35.4
sv^{na}	95	110	170	195	570	36.0
+, SanG.	64	125	111	157	457	41.4
+, Bham	66	84	60	140	350	42.8
bt	127	187	188	270	772	40.7
ey	71	89	77	115	352	45.5
+, Seattle	187	204	232	263	886	44.1
$gvl\ ey^R$	129	158	131	179	597	48.1
gvl	144	252	148	238	782	50.6
ey^4	101	137	56	98	392	60.7
ey^2	110	180	77	144	511	56.8

TABLE 4

Offspring of triplo-IV females of the type A/A/B, mated to BB males.

A	B	NOT B	B	TOTAL	PERCENT B
sv	$gvl\ ey^R$	181	74	255	29.0
sv	*gvl*	577	144	721	20.0
sv	ey^4	174	42	216	19.4
sv	ey^2	202	49	251	19.5
$ci\ ey^R$	sv^{na}	633	163	796	20.5
sv^{na}	$ci\ ey^R$	1227	183	1410	13.0
$ci\ ey^R$	*gvl*	791	243	1034	23.5
gvl	$ci\ ey^R$	771	92	863	10.7
$gvl\ ey^R$	$ci\ ey^R$	185	26	211	12.3
sv^{na}	$gvl\ ey^R$	976	213	1189	17.9
sv^{na}	*gvl*	1494	415	1909	21.8
sv^{na}	ey^4	1160	389	1549	25.1
ey^4	sv^{na}	659	58	717	8.1
sv^{na}	ey^2	440	155	595	26.0

are exceptions; here the value for *sv* is most aberrant. The explanation of this aberrant value is unknown, though it occurred in several independent tests and appears to be real. The general rule suggested by these results and by those of table 1 is: chromosomes high in the series tend to pass to the diplo-IV pole, those low tend to pass to the haplo-IV pole.

Table 4 shows the results from experiments of the type AAB, where the frequency of B eggs is determined. The frequency of this type of segregation is twice this value; the other two types of segregation are equivalent (AB/A), and their frequency may therefore be calculated $\left(=\frac{1-2B}{2}\right)$. Here

TABLE 5

Frequencies of types of segregation from triplo-IV females.

FEMALE AND TYPE OF SEGREGATION	NUMBER OFFSPRING	PERCENT	FEMALE AND TYPE OF SEGREGATION	NUMBER OFFSPRING	PERCENT
$sv\ sv/gvl\ ey^{R}$	255	58.0	$ey^{D}\ ci^{D}/bt$	772	40.7
$sv\ sv/gvl$	721	40.0	$ey^{D}\ ci^{D}/ey$	352	45.5
$sv\ sv/ey^{4}$	216	38.9	$ey^{D}\ ci^{D}/+$Seattle	886	44.1
$sv\ sv/ey^{2}$	251	39.0	$ey^{D}\ ci^{D}/gvl\ ey^{R}$	597	48.1
$sv\ ey^{2}/ci\ ey^{R}$	770	21.6	$ey^{D}\ ci^{D}/gvl$	782	50.6
$ey^{D}\ ci^{D}/sv$	1020	41.5	$ey^{D}\ ci^{D}/ey^{4}$	392	60.7
$sv\ gvl/gvl\ ey^{R}$	276	26.8	$ey^{D}\ ci^{D}/ey^{2}$	511	56.8
$sv\ ey^{2}/gvl\ ey^{R}$	493	23.5	$ci\ ci^{D}/ey^{4}$	249	48.2
$sv\ gvl/ey^{2}$	278	45.0	$sv^{na}\ sv^{na}/gvl\ ey^{R}$	1189	35.8
$sv\ ey^{2}/gvl$	278	35.2	$sv^{na}\ sv^{na}/gvl$	1909	43.5
$gvl\ ey^{2}/sv$	—	19.8	$sv^{na}\ sv^{na}/ey^{4}$	1594	50.2
$ci\ ey^{R}\ ci\ ey^{R}/sv^{na}$	796	41.0	$sv^{na}\ sv^{na}/ey^{2}$	595	52.1
$ci\ ey^{R}\ ci\ ey^{R}/gvl$	1034	47.0	$gvl\ ey^{R}\ gvl/sv^{na}$	573	25.4
$ey^{D}\ sv^{na}/ci\ ey^{R}$	341	28.2	$sv^{na}\ gvl/gvl\ ey^{R}$	1022	29.5
$ey^{D}\ ci^{D}/ci\ ey^{R}$	480	31.1	$sv^{na}\ gvl\ ey^{R}/gvl$	—	45.1
$sv^{na}\ sv^{na}/ci\ ey^{R}$	1410	26.0	$gvl\ ey^{R}\ ey^{4}/sv^{na}$	338	22.4
$ci\ ey^{R}\ bt/sv^{na}$	320	35.6	$sv^{na}\ ey^{4}/gvl\ ey^{R}$	361	30.4
$sv^{na}\ ci^{D}/ci\ ey^{R}$	820	23.9	$sv^{na}\ gvl\ ey^{R}/ey^{4}$	—	47.2
$sv^{na}\ bt^{D}/ci\ ey^{R}$	280	22.6	$sv^{na}\ gvl/ey^{4}$	398	35.2
$ci\ ey^{R}\ sv^{na}/gvl\ ey^{R}$	217	36.8	$sv^{na}\ ey^{4}/gvl$	398	32.6
$sv^{na}\ ey^{4}/ci\ ey^{R}$	1074	17.4	$gvl\ ey^{4}/sv^{na}$	—	32.2
$ci\ ey^{R}\ ci^{D}/gvl$	831	54.2	$ey^{4}\ ey^{4}/sv^{na}$	717	16.2
$ci^{D}\ gvl/ci\ ey^{R}$	831	19.1	$ci^{D}\ ey/gvl$	247	38.0
$ci\ ey^{R}\ gvl/ci^{D}$	—	26.7	$ci^{D}\ gvl/ey$	247	33.2
$bt^{D}\ ey^{4}/ci\ ey^{R}$	484	27.3	$ey\ gvl/ci^{D}$	—	28.8
$gvl\ ey^{R}\ gvl\ ey^{R}/ci\ ey^{R}$	211	24.6	$ci^{D}\ gvl/ey^{4}$	260	43.2
$ci\ ey^{R}\ gvl/gvl\ ey^{R}$	1852	36.1	$ci^{D}\ ey^{4}/gvl$	260	31.4
$gvl\ ey^{R}\ gvl/ci\ ey^{R}$	1110	25.6	$gvl\ ey^{4}/ci^{D}$	—	25.4
$ci\ ey^{R}\ gvl\ ey^{R}/gvl$	—	38.3	$ci^{D}\ gvl/ey^{2}$	763	37.4
$gvl\ gvl/ci\ ey^{R}$	863	21.4	$ci^{D}\ ey^{2}/gvl$	763	42.2
$ey^{D}\ ci^{D}/ci$	323	30.3	$gvl\ ey^{2}/ci^{D}$	—	20.4
$ey^{D}\ ci^{D}/+y\ sc\ w^{a}$	226	35.4	$ey\ gvl/gvl\ ey^{R}$	222	30.6
$ey^{D}\ ci^{D}/sv^{na}$	570	36.0	$gvl\ ey^{4}/gvl\ ey^{R}$	1174	25.2
$ey^{D}\ ci^{D}/+$San G.	457	41.4	$gvl\ ey^{2}/gvl\ ey^{R}$	1653	27.8
$ey^{D}\ ci^{D}/+$Bham	350	42.8			

there is no viability control, though in every case it may be surmised that the "B" class is slightly decreased from the true value by this factor.

Table 5 summarizes all the data available from triplo-IV experiments (of whatever type) in which as many as 200 flies were recorded. The final column gives the frequencies of the segregation-types concerned (that is, the values of table 3, twice those of table 4). These values have not been corrected, in cases where the viability correction is absent from the experiment itself; but the probability is that such corrections, were it possible to estimate them quantitatively, would be small in most cases.

Many of the values recorded in table 5 are strikingly different from the 33.3 percent that would result from random segregation. Analysis shows that these deviations are in general in agreement with the principle stated in discussing table 3. If A, B, and C represent the three chromosomes concerned, in the order of their position in the seriation, then the three types of segregation occur in the relative frequencies AB/C>AC/B>BC/A. More exact quantitative analysis will be discussed below.

ALGEBRAIC ANALYSIS OF THE DATA

Given three IV chromosomes (A, B, and C), the simplest assumption seems to be that when A and B pass to opposite poles at meiosis, C goes to the same pole as A in a fixed proportion of the eggs that is independent of the nature of C. It is clear, from even a cursory examination of the data, that the nature of C does influence the frequency with which A and B separate, so the absolute frequency of AC/B separation will be influenced by the nature of C; the assumption is that the nature of C does not influence the *relative* frequency of AC/B and A/BC.

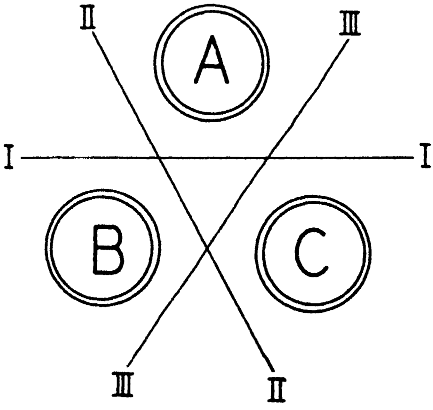

FIGURE 1. Three types of segregation of three IV chromosomes.

The testing of this assumption may be approached by an analysis of the three types of segregation into certain possible components. In figure 1 these three possible types are labelled I, II and III.

The terminology used follows:

Let $k = I + II$ (i.e., frequency of separation of A and B)
$l = I + III$ (i.e., frequency of separation of A and C)
$m = II + III$ (i.e., frequency of separation of B and C)

What will hereafter be referred to as the "preference ratios" may then be defined as follows:

r = proportion of k in which C goes with A $\left(\frac{II}{I+II}\right)$

s = proportion of l in which B goes with A $\left(\frac{III}{I+III}\right)$

t = proportion of m in which A goes with B $\left(\frac{III}{II+III}\right)$.

Then

$$I+II+III=1$$
$$I=l(1-s)=k(1-r)$$
$$II=kr=m(1-t)$$
$$III=ls=mt.$$

These equations may be shown to lead to the following results:

$$I=1-\left(\frac{r+s-2rs}{1-rs}\right) \qquad (1)$$

$$II=\frac{r-rs}{1-rs} \qquad (2)$$

$$III=\frac{s-rs}{1-rs} \qquad (3)$$

$$t=\frac{s-rs}{r+s-2rs} \qquad (4)$$

The first three numbered equations are of interest in dealing with triplo-IV cases; (4) has been used in the analysis of the sc^{10-2} duplication series. The original assumption was that the values of r, s, and t were independent of the nature of the third element concerned—i.e., that r is a constant property of the pair A B, whether they be tested against C, D, or Z. Therefore equation (4) furnishes a method of calculating the preference between

B and C when that between A and B and between A and C is known. The duplication series may be taken as giving such preference values directly, since one type of segregation is rare and does not enter into the calculations. If C (figure 1) be taken as the duplication, the rare type of segregation is III, and the definition of r is that it is equal to II/I+II. However, in most

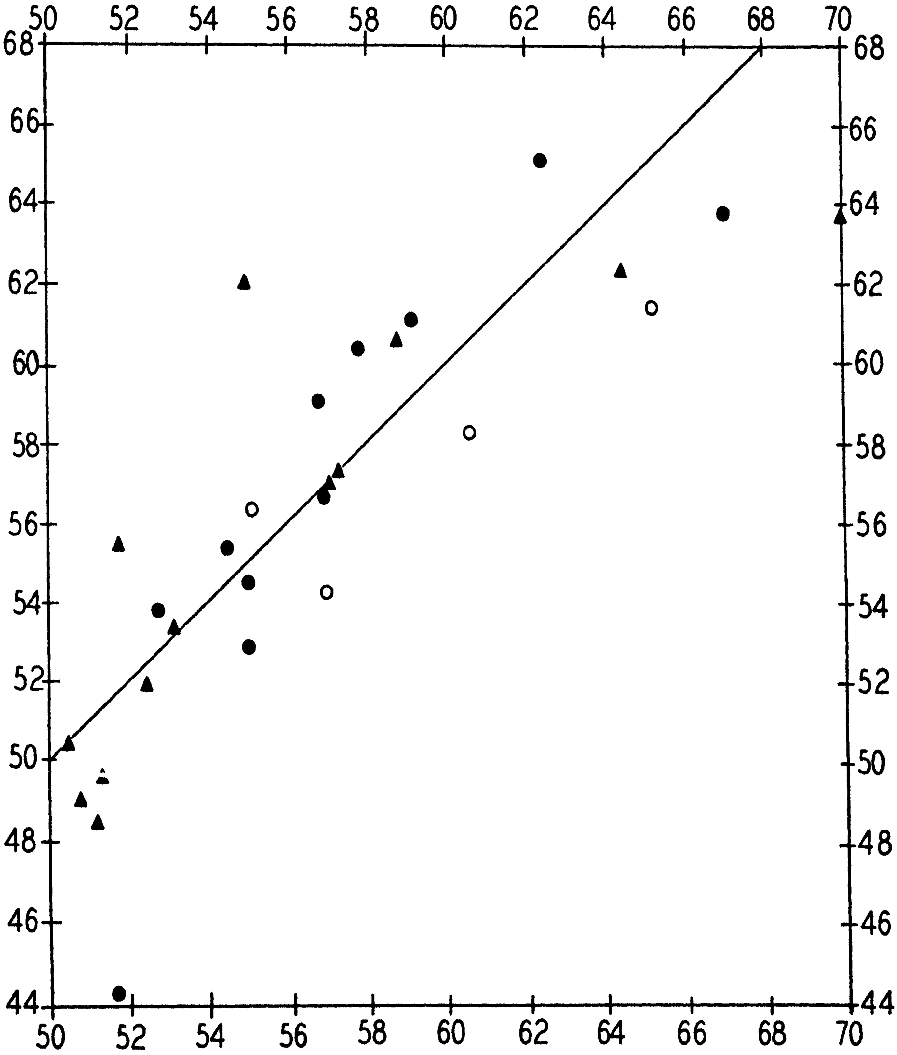

FIGURE 2. Comparison of observed (horizontal axis) and calculated (vertical axis) preference values for the scute-$^{10-2}$ duplication. Solid circles represent cases where the probable error of the observed value is 1.3 or less and the calculated value is an average of 9 or more determinations; solid triangles, cases where the probable error of the observed value is 1.3 or less, the calculated value the average of 5 to 7; open circles, cases where the probable error of the observed value is 1.4 or 1.5, the calculated value the average of 12 or more determinations.

cases the occasional occurrence of III will give rise to two classes, one of which will be indistinguishable from one of those resulting from I or II. Nevertheless, since III is infrequent, the data of tables 1 and 2 may be taken as giving direct determinations of preferences, and equation (4) may be applied to them. (See discussion below.)

Table 6 shows the calculated values of t for each of the 26 tested chromosomes in combination with the five most thoroughly tested chromosomes. (See column headings.) From tables 1 and 2 were extracted all cases in which pairs of chromosomes entered in the cells of table 6 were each tested against a common third chromosome. From such combinations t was calculated (for example, *sv*/ey^D gave 52.2, *ci* ey^R/ey^D gave 50.9; *sv*/*ci* ey^R may be calculated from equation (4), substituting 52.2 for r, 50.9 for s). The various possible calculated values for each cell of table 6 have been averaged; averages of this type have been used directly in figure 2, but in table 6 the observed values from tables 1 and 2 have been averaged in, each being given the same weight as a single calculated value.

TABLE 6

Average of calculated preference values (directly observed values also included), based on tables 1 and 2.

OPPOSED CHROMOSOME	PREFERRED CHROMOSOME				
	ci ey^R	ey^D	sv^{na}	*gvl* ey^R	*gvl*
+^{ci} ey^R	50.4	47.6	41.7	36.7	34.8
sv	48.1	49.6	44.9	36.0	36.4
ci ey^R	50.0	48.6	44.4	36.7	35.3
ey^D	51.4	50.0	45.5	38.1	36.0
ci	51.0	51.0	44.6	40.3	37.8
Xci	53.0	54.8	45.8	39.4	38.7
+*y sc* w^a	55.7	54.3	47.9	40.2	39.2
sv^{na}	55.6	54.5	50.0	41.2	39.6
+San G.	55.1	56.3	51.1	42.2	40.4
+Bham	57.2	58.5	52.9	44.3	42.7
bt	58.8	56.7	54.7	46.3	43.3
ci^D	59.2	58.4	54.4	45.5	43.7
+Tonto	58.2	60.9	52.1	45.9	45.0
bt^D	60.2	59.5	54.0	45.4	44.5
+Red. M	59.1	60.4	54.2	46.1	45.5
+Arr. S.	59.9	59.4	55.4	46.7	45.1
+Fla	60.5	61.3	53.6	46.7	45.3
ey	60.8	60.2	56.9	47.0	44.6
+Seattle	62.9	63.3	56.5	49.7	48.2
+Sch.X	62.4	63.2	57.2	50.2	47.8
gvl ey^R	63.3	61.9	58.8	50.0	47.2
ar	64.6	65.0	60.1	52.6	49.6
gvl	64.7	64.0	60.4	52.8	50.0
ey^4	67.1	66.1	62.2	53.8	50.8
M^4	67.7	67.3	64.0	57.7	51.0
ey^2	68.0	68.4	64.8	55.5	55.1

The values in this table are clearly self-consistent, each of the columns giving a regularly increasing series of values as one reads from top to bottom. The discrepancies that exist are all small in amount. In effect this table represents a "smoothing" of the raw data of tables 1 and 2. The regularity of the gradation of values is in part inevitable, since the final seriation is based on these data; the essential point is that it is possible to get so nearly a perfect gradation for all five columns simultaneously.

When either r or s is close to 50, equation (4) is approximated by the equation

$$t=50+r-s. \tag{5}$$

That is, t differs from 50 by an amount equal to r−s. This relation is useful in checking calculations; and has also been made use of in applying arbitrary numerical values to the various chromosomes in table 7. This table in appearance, in method of construction, and in practical application, has a resemblance to the familiar "chromosome maps" that are based on crossover values. It is necessary to keep in mind, however, that no spatial relations are represented, and that the table concerns properties of whole chromosomes, not of loci.

TABLE 7.

Preference table (Description in the text).

+''*ey*^{R}	20.6	+Bham.	11.2	+Seattle	6.3
sv	19.8+	*bt*	10.4	+Sch X	6.2+
ci ey^R	19.8	**ci**^{D}	10.1	**gvl ey**^{R}	6.2
ey^D	18.6	+Tonto	9.9	*ar*	4.2+
ci	17.4	*bt*^{D}	9.6	**gvl**	4.2
Xci	16.2	+Red. M.	9.3	*ey*^{4}	2.5
+*y sc w*^{a}	14.9	+Arr. S.	9.0	*M*^{4}	1.0
sv^{na}	14.2	+Fla.	8.8	**ey**^{2}	0.0
+San [illegible]	13.5	**ey**	8.4		

The table is based primarily on the relations of the eight most-studied chromosomes; these are represented in bold-faced type. The values of t for each successive pair of these were calculated from the values of table 6—i.e., "second order" calculations were made and averaged. The differences between these values and 50 were then taken as representing the differences between the chromosomes concerned. The interval concerned was first estimated by comparing the totals of the rows in table 6, and assuming the seriation to be such as to give a regularly ascending series of such totals. Each of the remaining 18 chromosomes was then fitted into the scheme by making "second order" calculations (from table 6) for its relations to the two of the chosen 8 better-studied chromosomes between which it lies. When the sum of the values so obtained was not equal to the difference between the two chosen chromosomes (in no case did it differ markedly) the fitting was done by the method of proportional parts. These calculations resulted in a seriation of the chromosomes, with a set of relative differences. One of the terminal chromosomes (*ey*^{2}) was arbitrarily taken as the zero point, and numerical values were assigned to each of the other 25 chromosomes, corresponding to the sum of the differences appearing in each interval between them and the arbitrary zero point.

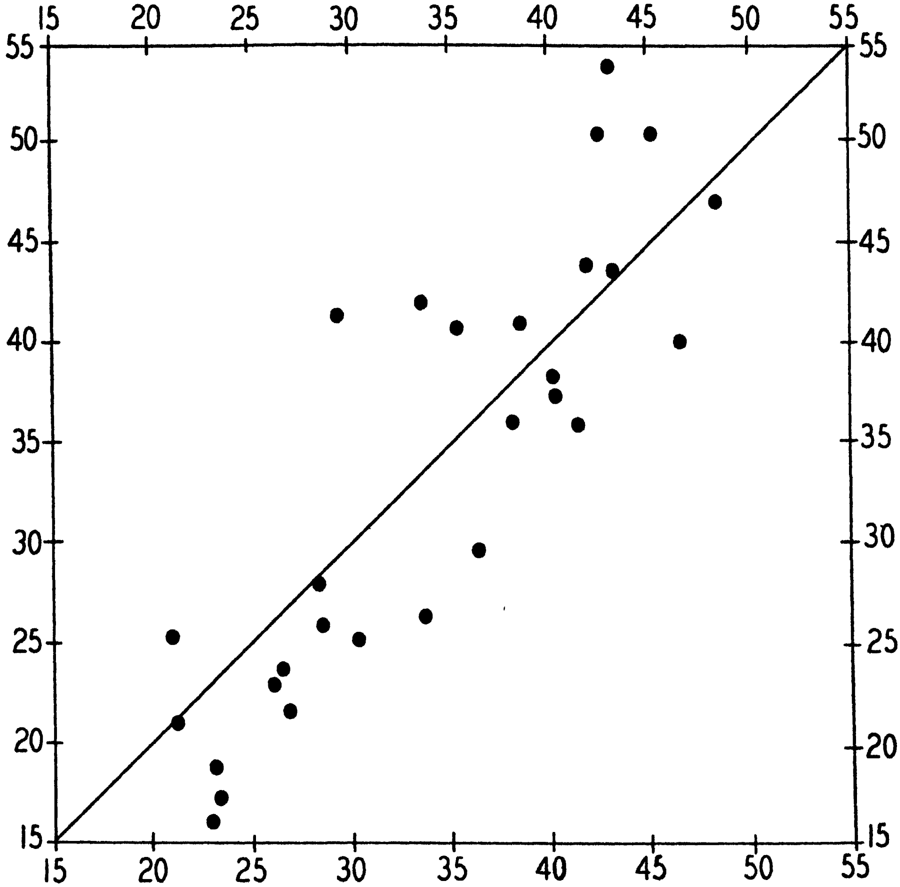

FIGURE 3. Comparison of observed (vertical axis) values for frequencies of segregation types in triplo-IV females with values calculated (horizontal axis) from the scute-$^{10-2}$ duplication experiments. Includes all cases where the observed value is based on 700 or more flies.

Table 7 may be used to give a prediction of the behavior of any three IV chromosomes when tested together. The differences between the values given in the table for any two chromosomes, when added to 50, give the preference ratio for those two. Three such values may be read off (for the possible combinations by two) for any three chromosomes; these, substituted for r, s, and t in equations (1), (2), and (3), give the required prediction. Furthermore, any IV chromosome not listed in table 7 may be entered in its proper place by means of very few (theoretically by any single one) combinations with known chromosomes; it will then be possible to predict its behavior with any listed chromosomes.

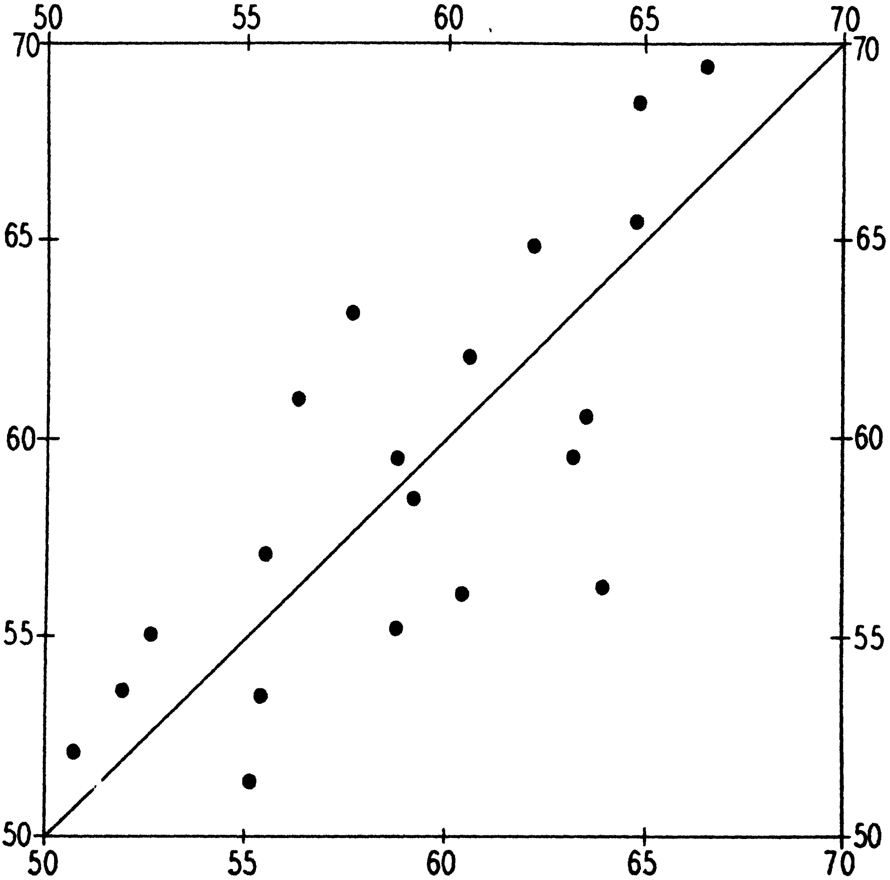

FIGURE 4. Preference ratios calculated from triplo-IV experiments (vertical axis) plotted against those calculated from scute-$^{10-2}$ duplication experiments (horizontal axis).

CORRELATION OF DUPLICATION AND TRIPLO-IV DATA

The equations developed in the preceding section were assumed to apply both to the duplication experiments and to those with triplo-IV females. It is possible to test this directly, by calculating from one series the results to be expected from the other. Figures 3 and 4 show the results of such a procedure.

Equations (1), (2), and (3) make it possible to calculate the types of segregation expected from triplo-IV females, given the preference ratios of table 6. In some cases the necessary values are not included in table 6; such values were calculated by the same method as that used in deriving table 6. The observed frequencies are recorded in table 5. Figure 3 shows the relations here; all cases where the observed value is based on 700 or more flies are entered. The agreement is evidently satisfactory—calculations based wholly on scute-10—2 duplication data do in fact give good agreement with observations on triplo-IV.

There is, however, a suspicion of a systematic deviation in figure 3, such that observation gives a greater deviation from randomness than calculation would lead one to expect. The problem was therefore approached in another way. From the definitions of r, s, and t it is possible to calculate them from triplo-IV data in cases where the frequencies of all three types of segregation are known. Table 5 includes a number of such cases. The preference values from these were calculated; where several determinations for the same two chromosomes were available these were averaged. The results are shown in table 8, and are plotted against the corresponding values of table 6 in figure 4. Here there is clearly no systematic deviation, so the indication of one in figure 3 is probably not significant. It may be noted that, if a table analogous to table 6 is constructed from the data of table 8, the same seriation of the nine chromosomes concerned will be

TABLE 8

Preference ratios calculated from triplo-IV data of table 5.

CHROMOSOMES		NUMBER OF DETERMINATIONS	AVERAGE PREFERENCE RATIO
sv	$gvl\ ey^R$	1	56.1
sv	*gvl*	2	60.5
sv	ey^2	1	69.4
$ci\ ey^R$	sv^{na}	2	57.0
$ci\ ey^R$	ci^D	1	58.3
$ci\ ey^R$	$gvl\ ey^R$	2	59.5
$ci\ ey^R$	*gvl*	4	65.4
sv^{na}	$gvl\ ey^R$	3	55.1
sv^{na}	*gvl*	3	56.0
sv^{na}	ey^4	4	64.8
sv^{na}	ey^2	1	68.5
ci^D	*ey*	1	53.5
ci^D	*gvl*	4	61.0
ci^D	ey^4	1	63.0
ci^D	ey^2	1	62.1
ey	*gvl*	1	53.4
$gvl\ ey^R$	*gvl*	2	55.0
$gvl\ ey^R$	ey^4	2	59.4
gvl	ey^4	1	51.9
gvl	ey^2	2	51.2

found to fit, with surprisingly few discrepancies and these of small magnitude. If the seriation is deduced solely from the data of table 8, it is found to agree with that shown in table 7, with the exceptions that *sv* might be placed below $ci\ ey^R$, *ey* below $gvl\ ey^R$ (only one comparison possible), and ey^4 below ey^2. In all these cases table 7 indicates only slight differences; the rearrangements suggested by the triplo-IV data would not seriously disturb them.

The calculations from the two series thus agree quantitatively as well as could be expected from the nature of the data.

INFLUENCE OF DUPLICATION ON SEGREGATION OF FOURTH CHROMOSOMES

It has been assumed, up to this point, that in diplo-IV females carrying the scute 10−2 duplication the frequency with which the two IVs go to one pole and the duplication goes to the other is negligible. It was clear, when the experiments of table 1 were carried out, that this type of segregation does occur; since these experiments regularly gave a few haplo-IV flies (more than the occasional ones found in any experiment), and such flies were always not-yellow (that is, carried the duplication). These flies were recorded, but have not seemed worth dealing with, since the viability of haplo-IV is extremely low and is also quite variable. In one of the experiments of table 1, however, it is possible to identify the other (triplo-IV) product of such segregation, namely in the combination $ey^D/ci^D/$dupl. From this combination there were recorded 146 ey^D, 106 ci^D, 94 $y\ ey^D$, 160 $y\ ci^D$, 11 $y\ ci^D ey^D$. The latter class results from the type of segregation under discussion; an equal number of (not-yellow) haplo-IV must have been produced but failed to survive. From these data one may deduce that the three types of segregation occurred with the following frequencies: ey^D dupl/$ci^D = 57.9$; ci^D dupl/$ey^D = 37.8$; $ey^D\ ci^D$/dupl $= 4.2$. It should be noted that the experiment suffers from the low viability of ey^D referred to before.

Several experiments were carried out in an attempt to determine the frequency of this type of segregation in another way. Females of the type studied in table 1 were mated to males of the constitution ey^D/M^4. M^4 is completely suppressed in triplo-IV flies; accordingly any wild type flies must have arisen by the production of a diplo-IV egg by the mother. Since both ey^D and M^4 have reduced viability, these experiments give maximum values, the haplo-IV eggs always giving less viable zygotes than the diplo-IV ones. The procedure adopted has been to double the number of + zygotes (since a corresponding number of haplo-IVs are lost), and add this number to that one of the two diplo-IV classes that was largest, in order to obtain a total number of zygotes. The data are given in table 9. The average value from this table is 4.55 percent non-disjunction, none having been found in the few offspring studied from females with no duplication. It may be noted that the highest frequency of non-disjunction was obtained from the combination involving the highest members of the IV chromosome seriation, as might have been expected. Taking this and the ey^D/ci^D/dupl experiment together, we may conclude that 4 percent represents a fair estimate of the usual frequency of this type of segregation.

TABLE 9

Diplo-IV females × ey^D/M^4 males

CONSTITUTION OF MOTHER	ey^D	M^4	+	CALCULATED NON-DISJUNCTION
sv/ey^4/dupl	90	67	1	2.2
+, $y\ sc\ w^a/sv^{na}$/dupl	129	99	6	8.5
sv^{na}/sv^{na}/dupl	204	231	7	5.7
sv^{na}/bt/dupl	102	100	3	5.6
+, SanG/ey^2/dupl	90	67	1	2.2
gvl/gvl/dupl	188	160	3*	3.1
Average	—	—	—	4.55
sv/ey^4	191	149	0	0
sv^{na}/sv^{na}	85	80	0	0

* These flies, being $gvl/gvl/M^4$, were expected to show the grooveless character, as do gvl/M^4 flies. No trace of grooveless was found; the constitution of one of the three flies was verified by tests of its offspring. This result is presumably related to the fact that the grooveless character is often slight in haplo-IV *gvl*.

If 4 percent is taken as the frequency of AB/dupl segregation, it is possible to estimate the error introduced by its occurrence in the experiments of tables 1 and 2. If A and B each carry a recessive gene (*a*, *b*), the calculated preference will differ according as the male used in the test is *y a* or *y b*. If r represents the true preference ratio (for A over B), then in backcrosses to *y a* males the observed ratio will be r+2/102; in backcrosses to *y b* males it will be r/102. These expressions give the following values:

Male	r = 50	r = 60	r = 65	r = 70
y a	51.0	60.8	65.7	70.6
y b	49.0	58.8	63.7	68.6

Table 10 shows the results from all experiments in which females of a given constitution were mated to both kinds of males. The average difference between the two types of experiments, instead of being 2.0 as expected, is only 0.2; evidently this source of error in tables 1 and 2 is too slight to be further considered.

POSITION OF THE DUPLICATION IN THE SERIATION

It is clear that the duplication may be placed in the same seriation as the other IV chromosomes. Since, as just shown, it almost always goes to the diplo-IV pole, it belongs at the top of the series, far above the other chromosomes dealt with. There is too much uncertainty about the values to enable one to place it numerically in relation to table 7.

TABLE 10

Females A/B/dupl mated to males y a or y b.

a	*b*	BACKCROSS TO *a*		BACKCROSS TO *b*		*a* MINUS *b*	
		N	CALC. PREFERENCE	N	CALC. PREFERENCE		
$ci\ ey^R$	*gvl*	625	67.2	583	68.5		−1.3
$ci\ ey^R$	sv^{na}	1979	56.0	361	55.1	+0.9	
gvl	ey^2	821	48.2	203	50.8		−2.6
gvl	ey^4	378	50.2	587	48.6	+1.6	
ey	*gvl*	200	58.0	802	54.6	+3.4	
Xci	sv^{na}	505	52.3	216	48.1	+4.2	
sv^{na}	*ey*	149	53.6	532	57.5		−3.9
sv^{na}	ey^2	201	64.1	306	65.4		−1.3
sv^{na}	*gvl*	174	58.6	678	57.5	+1.1	
				Average		+0.23	

IV CHROMOSOME TRANSLOCATIONS

Several reciprocal translocations between the IV and the II or III chromosomes have been tested for preference. They have not given consistent results, presumably because in all such cases there are types of gametes that are not recovered (or have greatly reduced viability). Until these complications can be eliminated it does not seem necessary to present what data were obtained.

NO INFLUENCE OF AGE AND TEMPERATURE

Two separate experiments were carried out to test the effects of age of female and of temperature on the preference ratios. In the first case the tested females were sv/ey^D, carrying the scute 10−2 duplication. Here controls (25°) and females treated for two days at 27°C immediately after emergence showed no clear differences from the usual value of 52 percent in daily transfer cultures run for 12 days. In the second series the females were ci^D/gvl, with the duplication. The temperatures used were 19°, 25°, and 28°; the first and last were applied for three days that included the emergence from the pupae. Again there were no evident deviations from the usual value (55 percent) in daily transfer cultures made for 12 days. These temperatures and ages are evidently without effect on the preference ratios.

PREFERENCE TESTS IN MALES

The results described in the preceding pages have been based entirely on tests of females. The results obtained from tests of males are shown in tables 11 and 12. Table 11, dealing with scute-10—2 duplication males, suggests that there is no preference in the male, none of the values being clearly different from randomness (50.0). However, it is to be noted that the four combinations that give over 66.0 in females all give above 50.0 in males, while the four that give 57.0 or less in females all give less than 50.0 in males.

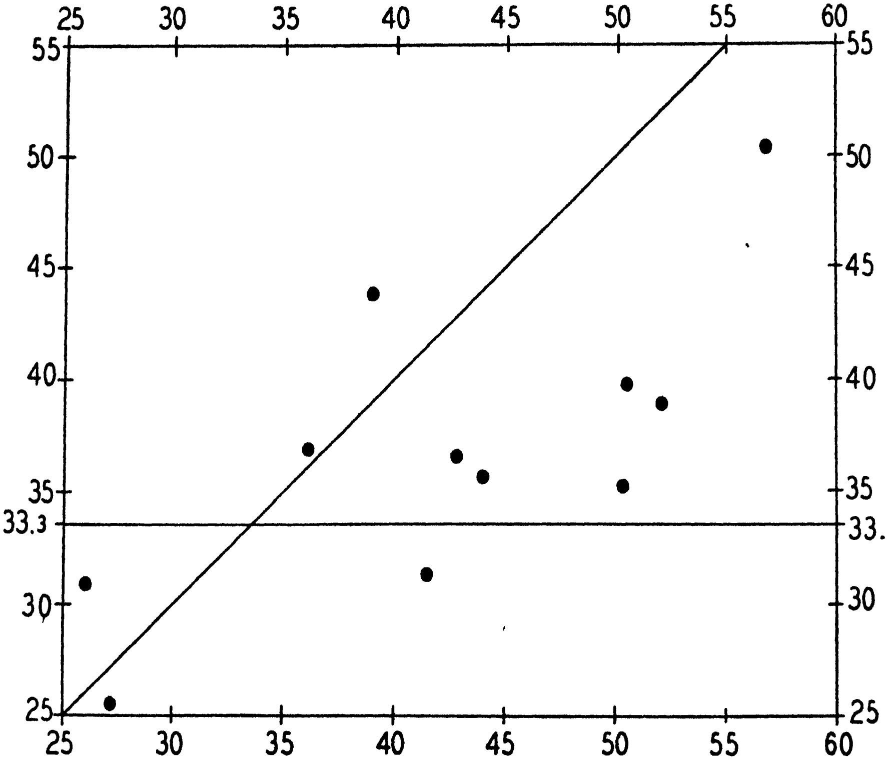

FIGURE 5. Frequencies of segregation types in triplo-IV males (vertical axis) plotted against the corresponding frequencies from triplo-IV females (horizontal axis).

Table 12, showing the results from triplo-IV males, indicates the existence of a preference in males that is less than that in females. The correlation between comparable values for the two sexes is obvious in figure 5. The numbers of combinations are too few to warrant presentation of the results of application of the preference formulae to males; the deviations from randomness are so slight that very large numbers would be required to furnish an adequate test of these formulae.

PREFERENCE OF THE DUPLICATION WITH RESPECT TO X AND Y

Tables 11 and 12 suggest that preference is more marked in triplo-IV males than in diplo-IV males carrying scute-10—2 duplication. One interpretation of this result might be that the unpaired X, having genes in common with the duplication, affects its behavior by reducing its dependence on IV. If this were correct one might expect a relation of the duplication to the X-Y segregation in such males. The available data on this relation follow.

TABLE 11

Preference values for the scute-10—2 duplication in males and in corresponding females

PREFERRED CHROMOSOME	OPPOSED CHROMOSOME	VALUE OBSERVED	
		MALE	FEMALE
$ci\ ey^R$	ar	52.7±1.6	70.0±1.3
sv^{na}	bt^D	49.5±1.4	56.6±1.7
sv^{na}	ci^D	49.2±2.1	57.0±1.4
$ci\ ey^R$	gvl	52.6±0.8	67.8±0.9
ey^D	gvl	52.0±1.1	67.0±0.7
$ci\ ey^R$	sv^{na}	49.7±1.7	56.8±0.7
ey^D	sv^{na}	46.3±2.1	55.0±1.2
sv	ey^4	50.8±1.9	66.4±1.9

TABLE 12

Frequencies of segregation types in triplo-IV males and females compared.

	MALES		FEMALES	
	OFFSPRING	PERCENT	OFFSPRING	PERCENT
$sv\ sv/ey^2$	488	43.8	251	39.0
$ey^D\ ci^D/sv$	346	31.3	1020	41.5
$bt^D\ ey^4/ci\ ey^R$	343	25.6	484	27.3
$sv^{na}\ sv^{na}/ey^4$	300	35.4	1594	50.2
$ey^D\ ci^D/sv^{na}$	597	36.8	570	36.0
$ey^D\ ci^D/$+Bham.	382	36.7	350	42.8
$ey^D\ ci^D/$+Seattle	639	35.7	886	44.1
$ey^D\ ci^D/ey^2$	253	50.5	511	56.8
$ey^D\ ci^D/gvl$	384	39.7	782	50.6
$sv^{na}\ sv^{na}/ci\ ey^R$	592	31.1	1410	26.0
$sv^{na}\ sv^{na}/ey^2$	704	38.9	595	52.1

Male *y*, carrying scute-10—2 dupl

	$+^y$ ♀	*y* ♀	$+^y$ ♂	*y* ♂	Total
♀ *yy* (X's separate)	854	1010	772	1031	3667
♀ $\bar{y}\bar{y}$ (X's attached)	252	350	314	368	1284

Analysis shows that in both series the duplication more often goes to the same pole as the Y (51.4 ± 0.6 percent and 51.7 ± 0.9 for the two series, 51.5 ± 0.48 for the two taken together). The deviation from randomness is very slight, and is doubtfully significant; since it is in the same direction in both series I am inclined to suppose that it is real.

Nevertheless the suggestion that such a relation is responsible for the difference between triplo-IV and duplication males cannot be taken as verified; for there is a more marked relation of the duplication to the sex-chromosomes in attached-X females, where its preference ratios for IV are unaffected. The data on this relation are as follows: $\bar{y}\bar{y}$ dupl ♀ crossed with *y*♂ gave + ♀ 1914, *y* ♀ 1793, + ♂ 1872, *y* ♂ 2162; preference of duplication for $\overline{XX}$ (as opposed to Y) = 4076/7741 = 52.6 ± 0.38 percent. That the result is not due to viability complications is shown by two facts. First, similar experiments using females with separate X's gave the same four classes as above in the frequencies 1099; 1178; 939; 1076. Here there can be no question of preference, since the mothers carried the duplication, whereas the fathers were responsible for the sex of the offspring. The percentage corresponding to the preference ratio above must then be 50.0 except for deviations due to viability. It is 50.6 ± 0.52. Second, other duplications, that have X spindle-attachments, similar in gene-content to the scute-10–2 duplication, give similar preferences for $\overline{XX}$ as opposed to Y. My own observations on duplication 118 give 307/555 = 56.4 ± 1.4; the data published by DOBZHANSKY (1934, p. 149) show similar relations for duplications 101, 106, 107, 118 and 136. The interpretation of these relations awaits further experiments.

DISCUSSION

The algebraic analysis of the data from triplo-IV females given above has been tested and shown to be adequate. However, the IV chromosomes concerned are cytologically visible bodies, and a satisfactory description of their behavior must have a geometrical basis.

In the earlier account (STURTEVANT 1934) it was indicated that 66.7 was the limiting preference ratio, and that an interpretation might be approached from this observation as a starting point. The more extensive data here presented do not bear out the suggestion of such a limiting value; tables 1 and 6 suggest rather that the value is slightly exceeded and that there is no approach to a limit. If the duplication itself be considered as a IV chromosome, then the preference ratio for it as compared to any other IV chromosome is nearer 90 than 66.7.

One natural assumption is that two of the IV chromosomes pair and pass to opposite poles, leaving the third to go to either pole at random. The preference seriation would then be an indication of the strength of pairing affinity. This hypothesis alone is inadequate; for if, in *ABC*, pairing is always between *A* and *B*, there will result two types of segregation in equal numbers, and this value of 50 percent is the maximum that can be obtained. Since 50 percent is clearly exceeded in most of the duplication experiments and in several of those with triplo-IV, this hypothesis alone is inadequate.

Perhaps the most attractive of the remaining possible hypotheses is that all three chromosomes conjugate, as in figure 1, and preference is due to non-random orientation of the resulting hexad on the first meiotic spindle. One may suppose that the orientation is always such that one chromosome is directed toward one pole of the spindle, two toward the other. On this basis, a chromosome near the bottom of the preference seriation is one that is more likely to be directed toward a pole; it may be looked upon as being in some sense the equivalent of more than a single one of any type of chromosome lying above it in the seriation. It is probable that a workable model of this type could be imagined in terms of electrical charges.

The scute-10–2 duplication probably has nothing in common with the other IV chromosomes except its inert material and its spindle-attachment. The data on crossover IV chromosomes (of which more are needed) suggest also that these regions of normal IVs may be especially important in determining preference properties. Since the segregation relations of X and Y are also related to inert material and spindle-attachments, it seems likely that they are essential in the relations described in this paper. The spindle-attachment alone cannot be solely responsible, since the crossovers show that more than one locus must be involved. Since BRIDGES (1935) has shown that there is inert material at both ends of the IV chromosome, this demonstration does not weaken the argument for its importance.

SUMMARY

1. Segregation in triplo-IV females of *Drosophila melanogaster* usually gives two chromosomes to one pole, one to the other.

2. The three resulting types of segregation do not usually occur with equal frequencies.

3. The IV chromosomes studied may be arranged in a definite seriation, such that any chromosome will, in a triplo-IV female, pass to the haplo-IV pole more often than will any chromosome that lies above it in the seriation (when the two are tested in like experiments or in the same experiment).

4. If two chromosomes, *A* and *B*, are tested against any third chromosome, *C*, then in those cases in which *A* and *B* separate, *C* will go with *A*

in a proportion of the cases, r, that is independent of the nature of *C*—that is, r is a constant property of the pair *AB*.

5. If the preference ratio for *A* as opposed to *B* is called r, that for *A* as opposed to *C* is called s, and that for *B* as opposed to *C* is called t, then

$$t=\frac{s-rs}{r+s-2rs}.$$

6. From this and related equations it is possible to show that the data form a consistent whole, and to predict the results that will be given by any combination of three tested chromosomes.

7. The segregation in triplo-IV males is also non-random. It deviates from randomness in the same direction as that in corresponding females, but less markedly.

8. More than one locus is concerned with the preference properties of a IV chromosome.

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EFFECTS OF TEMPERATURE ON FERTILIZATION IN HABROBRACON

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INTRODUCTION

IN THE parasitic wasp *Habrobracon juglandis* (Ashm.), when a cross of unrelated stocks is made, males (haploid) come from unfertilized eggs and females (diploid) from fertilized eggs. When the parental stocks are closely related, not only males from unfertilized eggs but also males (diploid) from fertilized eggs appear. That such biparental males should be produced was puzzling until WHITING brought forth his theory of sex determination in the Hymenoptera (1933a, 1933b, 1935).

According to this theory females have the sex chromosomes X and Y, while haploid males have either X or Y and biparental males either two X's or two Y's. One might then expect equal numbers of biparental male and female offspring according to the principle of random fertilization. However the number of biparental males is always much smaller than that of the females. This deviation from equality WHITING has explained in part by differential mortality, in part by differential maturation. When parental stocks are unrelated differential maturation is complete, all zygotes being XY and resulting in females only. Due to the greater viability of females in contrast to that of diploid males, hatchability of eggs and viability of offspring is much greater when parents are unrelated. This greater fecundity together with lack of biparental males, necessitates the supplementary hypothesis of differential maturation.

It has already been shown (WHITING and ANDERSON 1932) that temperature also affects the production of biparental males among progeny from crosses of related stocks. A much higher percentage of males among biparentals was produced when mothers were set at high temperature (30°C) than at low (20°C), while the percentage of biparentals, males and females, among total offspring was decreased.

After BOSTIAN (1934) produced an orange-eyed stock (11-o) closely related by grading to a certain wild-type stock (11) and giving very high percentages of males among the biparentals, it was shown (ANDERSON 1935) that if stock 11 males were reared at different temperatures (20°, 30°, 36°C) even though the stock 11-o females were kept at constant temperature (30°C), significant differences appeared in percentages both of males among biparentals and of biparentals among total offspring. In this case the differences must have been due, not to differential viability of heterosyngamic (XY), homeosyngamic (XX and YY) and unfertilized (X and Y) eggs, but to change in percentage of eggs fertilized and of type of fertilization.

EFFECTS OF CULTURE TEMPERATURES

Preliminary experiment. Females of stock 11-o and males of stock 11 were mated at room temperature and the females were then divided into three groups. One group was set at 32°C, a second at 27°C, and a third at 20°C. As usual the females were transferred through vials a, b, c, d and e when larvae appeared. This was every four days for the 32°C and the 27°C groups, but every ten or twelve days for those at 20°C. No transfer was made to a vial e in the low temperature group.

As in our previous experiments, the higher the temperature the greater is the production of males among biparentals while percentage of biparentals is reduced (table 1). In all three groups (table 2) there is a gradual rise of males among biparentals from vials a through b to c and a slight drop from c to d. In the high temperature groups a very significant rise occurs from vials d to e.

TABLE 1

Stock 11-o females by stock 11 males: preliminary experiment. Totals and percentages with standard errors for the different culture temperatures.

GROUP	TEMPERATURES	BIPARENTAL		IMPATERNATE	PERCENTAGE OF MALES AMONG BIPARENTALS	DIFFERENCES		PERCENT AGE OF BIPARENTALS	DIFFERENCES	
		♀♀	♂♂	♂♂						
A	32°C	703	242	489	25.61 ± 1.42	A-B	5.44 ± 1.78	63.75 ± 1.27	A-B	2.15 ± 1.62
B	27°C	1112	281	792	20.17 ± 1.07	B-C	7.62 ± 1.97	65.90 ± 1.01	B-C	6.54 ± 2.14
C	20°C	356	51	155	12.56 ± 1.64	A-C	13.05 ± 2.22	72.44 ± 1.88	A-C	8.69 ± 2.27

Egg-counting experiment. As a further check on temperature effects, an experiment was undertaken in which eggs were counted according to methods developed by ANNA R. WHITING and by C. H. BOSTIAN. In much of the tedious technical work involved in this research the writer was greatly assisted by Miss Roger Young, to whom he wishes to express his sincere gratitude.

Stocks 11 and 11-o were used and matings were made at room temperature of males and females reared at 30°C. Sister females were mated to the same males, half of the females mated to each male being set at lower temperature (18°–20°C) and half at higher temperature (32°–33°C). The females were given caterpillars that had been stung by wasps with mutant factors other than orange and the paralyzed caterpillars were also carefully examined and freed from any eggs observed that were laid by the "foster mothers." The females were then set in the incubators.

For the first four days each wasp was given one stung caterpillar each day. On the fifth day each female was given two caterpillars. The number of eggs laid by the females at high temperature was greatly increased by this increased feeding, but the additional caterpillar had no effect at the lower temperature. An increase to three caterpillars made no noticeable difference in the number of eggs laid at either temperature. Each caterpillar was placed on a glass slide in the desired position and covered by a small dish containing the female wasp. When observations were made the slide was put on a glass stage of a low power binocular microscope under which was placed a mirror so that the eggs on the under surface could be counted by focussing down on the image. In no case was it necessary to move the caterpillar and thus endanger the results by shaking off the eggs. All egg counts were made in rooms held at temperatures to correspond to those of the incubators in which the eggs were being developed.

TABLE 2

Biparental offspring from stock 11-o females by stock 11 males: preliminary experiment. Summaries and percentages according to culture temperatures and ages of mothers (vials).

TEMPERATURE	VIALS	BIPARENTAL OFFSPRING ♀♀	BIPARENTAL OFFSPRING ♂♂	PERCENTAGE OF MALES AMONG BIPARENTALS
32°C	a	187	56	23.04
	b	252	86	25.44
	c	149	55	26.96
	d	63	23	26.74
	e	52	22	29.80
27°C	a	267	55	17.08
	b	288	62	17.71
	c	299	87	22.52
	d	166	46	21.69
	e	92	31	25.20
20°C	a	112	12	9.64
	b	137	16	10.46
	c	41	9	18.00
	d	66	14	17.50
Totals	a	566	123	17.85
	b	676	164	19.52
	c	489	151	23.59
	d	295	83	21.96
	e	144	53	26.90

The eggs and the larvae for the high temperature group were counted every day until pupation occurred. Eclosion at this temperature took place seven and a half days after the wasps were set with the caterpillars.

For the cool temperature group the eggs were counted on the first day after setting and the females were transferred to new caterpillars. At this low temperature the larvae did not begin to appear until the fourth day. In general if the eggs had not developed into larvae by the sixth day, they failed to develop at all. The larvae were then counted on the fifth and sixth days and checks were made on them every other day until eclosion. Eclosion began about the twenty-seventh day.

In the cool temperature material after the seventh or eighth day, there were noticed several larvae which failed to increase in size and soon died. Although such inviable larvae were not found in the high temperature material developing at a rate almost four times as fast, they may nevertheless have been present. The higher percentage of eggs producing larvae and the lower percentage of larvae producing pupae recorded for the low temperature material (table 3) may be thus explained.

TABLE 3

Stock 11-o females by stock 11 males: egg-counting experiment. Totals, fecundity averages and viability percentages with standard errors at different culture temperatures.

Temperatures	32–33°C	18–20°C
Number of females	11	14
Days set	92	284
Eggs	1839	1990
Larvae	1284	1624
Pupae	1048	1227
Biparental females	428	532
Biparental males	152	67
Impaternate males	321	508
Egg production per day	20−	7+
Percentage of eggs producing larvae	69.82±1.07	81.61±0.87
Percentage of larvae producing pupae	81.62±1.78	75.25±1.05
Percentage of pupae producing adults	84.06±1.13	90.59±0.85
Percentage of eggs producing adults	48.98±1.16	55.63±1.11
Percentage of eggs producing females	23.22±0.97	26.73±0.98
Percentage of eggs producing biparental males	8.26±0.72	3.37±0.47
Percentage of eggs producing impaternate males	17.45±0.87	25.53±0.98
Percentage of males among biparentals	26.21±1.83	11.18±1.28
Percentage of biparentals	64.37±1.59	54.11±1.50

In this experiment we find the percentages of males among biparentals at high and at low temperatures to be approximately the same as in our preliminary experiment. The percentages of biparentals are reversed, however, being higher in the high temperature group than in the low. This is probably due to the smaller number of impaternate males surviving at the higher temperature, percentage of eggs producing impaternate males. The high temperature, being slightly higher than in the preliminary experiment, may have reduced the viability of the males as has been previously shown (WHITING and ANDERSON 1932).

The percentage of eggs developing into biparental adults, both males and females, is but slightly higher (31.48 percent) in the high temperature material than in the low (30.10 percent). Nevertheless the percentage developing into biparental males is almost two and one half times as great. Evidently homeosyngamic fertilization rather than heterosyngamic is favored by higher temperature. If it be supposed that higher temperature is differentially deleterious to the males among the biparentals, then homeosyngamy must be favored even more than the percentages indicate.

WHITING (1935) suggested sex-reversal of the XX or YY combinations into females as an alternative to the hypothesis of differential maturation but did not think this at all probable, nor did he suggest any explanation for such a reversal. SNELL (1935) proposed a multiple chromosome hypothesis according to which XX or YY might develop into females if heterozygous for any other sex-chromosome pair, ZW for example. The facts herewith reported cannot be explained by the multiple chromosome hypothesis since the material used was genetically homogeneous.

SUMMARY

A preliminary experiment with crosses of closely related stocks of Habrobracon showed an increase in males among biparental offspring but a decrease in biparentals as culture temperatures were increased. At all temperatures percentages of males among biparentals changed significantly with increasing age of mothers.

An experiment in which eggs were counted showed that at the higher temperature the percentage of eggs producing impaternate males was decreased. Among biparental offspring males were increased and females correspondingly decreased.

The conclusion is drawn that increased temperature increases mortality of males and increases homeosyngamy (male-producing combinations, X with X or Y with Y) at the expense of heterosyngamy (female-producing, X with Y).

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GEOGRAPHICAL DISTRIBUTION AND CYTOLOGY OF "SEX RATIO" IN DROSOPHILA PSEUDOOBSCURA AND RELATED SPECIES

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INTRODUCTION

A gene has been found in wild populations of several related species of Drosophila, which causes any male which carries it to produce few or no sons. The cytological analysis has shown why this is so, and also bears on several problems in chromosome behavior. The gene is widely distributed, both geographically and taxonomically, and raises a number of problems in population mechanics.

HISTORICAL

In 1922 one of us found (MORGAN, BRIDGES, STURTEVANT 1925) a strain of *Drosophila affinis* in which occasional males produced families consisting almost entirely of females. The data suggested that this peculiarity was due to the nature of the X chromosome carried by such a male, but the strain was lost before a detailed study could be carried out.

GERSHENSON (1928) later found a similar gene in the European *Drosophila obscura*, and made a careful analysis. He showed that the gene is in the X, and that a male carrying it produces very few sons, regardless of the nature of his mate. Females homozygous or heterozygous for the gene give normal (1:1) sex ratios when mated to normal males, and are fully fertile. Mated to males carrying the sex ratio gene they give the same excess of daughters as do normal females in these circumstances. Egg counts showed that the "sex ratio" result is not due to the death of male zygotes, since there is no greater mortality from such cultures than from controls giving a 1:1 sex ratio. The "sex ratio" gene thus has the effect of causing a male that carries it to produce nearly all X sperm, instead of the usual 50 percent.

GERSHENSON found the "sex ratio" gene to be present (heterozygous) in two of nineteen wild females studied. He pointed out that the gene should automatically increase in frequency in any population, since a male that receives it from only one parent transmits it to nearly all his offspring, while in females it is transmitted like any other gene. In fact such an increase does not occur, but the reason for this is unknown.

In 1929 this same gene, or at least a gene with the same properties, was found to be present in wild *Drosophila pseudoobscura* near Pasadena. The

TABLE 1

Tested X chromosomes in wild females (N—normal, S—sex ratio)

LOCALITY	YEAR	N	S
Race A			
British Columbia			
Shuswap Lake	1934	3	0
Arrowhead	1934	4	0
Nakusp	1934	5	0
Kaslo	1934	2	0
Washington			
Metaline Falls	1934	7	0
Idaho & Montana			
Lake Coeur d'Alene	1934	11	0
Bitterroot Mountains	1934	3	0
Oregon			
Days Creek	1933	2	0
Wyoming			
Big Horn Mountains	1935	3	0
South Dakota			
Black Hills	1935	4	0
Nebraska			
Scottsbluff	1935	4	0
California			
Mount Lassen	1934	3	0
Lake Tahoe	1935	2	0
Sequoia Nat. Park	1933	6	0
Santa Lucia Mountains	1934	49	1
Pasadena	1932	2	0
Pasadena	1933	5	3
Pasadena	1935	14	3
Fish Canyon	1930	5	3
Big Bear Lake	1932	4	1
Big Bear Lake	1934	12	0
Corona del Mar	1932	3	1
Henshaw Lake, near San Diego	1934	7	1
Nevada			
Charleston Peak	1935	3	2
Utah			
Cedar City	1934	2	0
Cedar City	1935	4	0
Zion Nat. Park	1934	2	2
Zion Nat. Park	1935	2	2
Bryce Nat. Park	1935	6	0
Northern Arizona			
Grand Canyon	1935	5	0
Flagstaff	1935	4	2
Southern Arizona			
Santa Catalina Mountains	1935	8	4
Santa Rita Mountains	1935	10	2
Chiricahua Mountains	1935	2	4
Race A			
Colorado (North)			
Aspen	1934	2	0
Estes Park	1935	5	0
University Camp	1935	4	1
Pikes Peak (slope)	1935	5	1
Pikes Peak (tree line)	1935	5	1
Colorado (South) & New Mexico (North)			
Mesa Verde Nat. Park	1935	5	1
San Juan Mountains	1935	6	0
Zuni Mountains	1935	4	2
Taos	1935	3	0
Southern New Mexico			
Pinos Altos Mountains	1935	4	2
Magdalena	1935	3	3
Carizozo	1935	4	2
Mexico			
Otinapa, Durango	1935	1	1
Cuernavaca, Morelos	1935	6	2
Cerro San Jose, Oaxaca	1935	4	0
Race B			
British Columbia			
Quesnel	1934	1	0
150-mile House	1934	1	0
Oregon			
Newport	1933	8	0
Reedsport	1933	13	1
Gold Beach	1933	11	3
Days Creek	1933	4	0
Oregon Caves	1933	4	2
California			
Klamath	1933	14	6
Eureka	1933	12	3
Shelter Cove (Humboldt)	1933	10	2
Mendocino	1933	4	0
Mount Lassen	1934	5	2
Lake Tahoe	1935	1	0
Sequoia Nat. Park	1933	7	3
Santa Lucia Mountains	1934	8	0

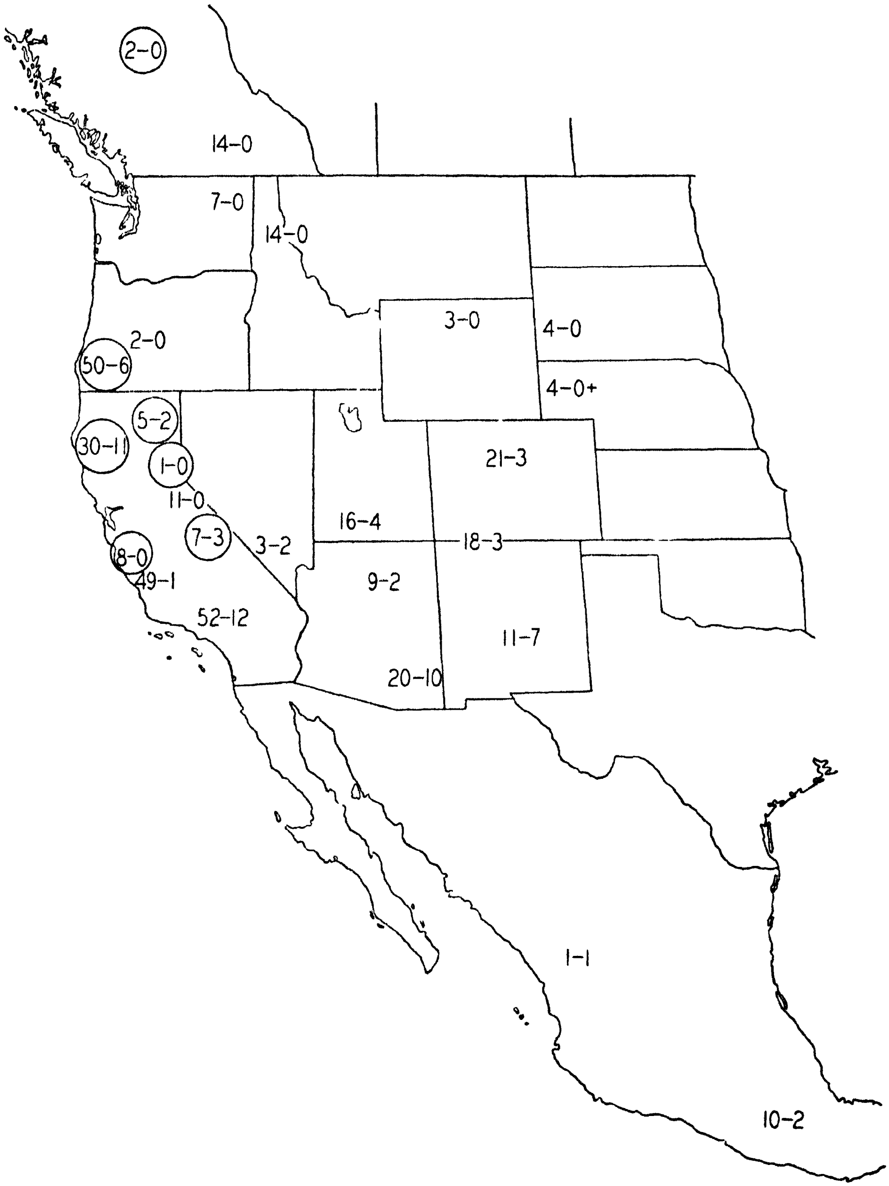


FIGURE 1. Geographical distribution of "sex ratio" factors. In each pair of figures the first indicates the number of tested X chromosomes in which no "sex ratio" was present, and the second shows the number of X's containing "sex ratio." Figures in circles—race B, figures without circles—race A.

present paper gives the results of our study of the geographical distribution and the cytological properties of this form, based chiefly on *D. pseudoobscura*. We have also made additional observations on *Drosophila affinis* and on two undescribed species near *D. affinis* in which "sex ratio" also occurs.

GEOGRAPHICAL DISTRIBUTION OF "SEX RATIO" IN DROSOPHILA PSEUDOOBSCURA

Tests soon showed that the "sex ratio" gene is present in both races (A and B) of *D. pseudoobscura*. Table 1 and figure 1 show its range. These data are based on tests of individual sons of females that were trapped out-of-doors. The attempt was made to test at least five sons from each female studied; in many cases fewer tests were actually made. The following convention was adopted: if both kinds of sons (normal and "sex ratio") were recovered, whatever the total number tested, it was considered that both X's of such a female were tested; if two were tested and were found to be alike it was considered that only one of her X's was tested; if three or more like sons were found it was assumed that both X's of the mother were tested. Other kinds of tests have shown the existence of sex ratio in a few other localities not shown in table 1: one of the wild females collected at Scottsbluff, Nebraska (Race A), and one of those at Days Creek, Oregon (Race B), had mated in nature with a sex ratio male and gave a typical "sex ratio" result. These, together with the records from wild males (table 2) have not been included in the map (figure 1), since they are not directly comparable quantitatively with table 1.

TABLE 2

Tested wild males (N—normal, S—sex ratio)

LOCALITY	YEAR	N	S	LOCALITY	YEAR	N	S
Race A				Santa Lucia Mts.	1934	45	2
California				*Utah*			
Big Bear Lake	1932	9	0	Bryce Nat. Park	1934	1	0
Big Bear Lake	1934	10	0	Cedar City	1934	3	0
Claremont	1932	2	0	Zion Nat. Park	1934	1	1
Fish Canyon	1930	3	1	*Nevada*			
Pasadena	1932	8	3	Las Vegas	1935	3	0
Arroyo Seco	1935	3	0	*Arizona*			
Henshaw Lake	1934	1	1	Gila, near Yuma	1935	0	1
Providence Mts.	1935	1	0	*Race B*			
Upper Kern Valley	1934	1	0	Upper Kern Valley, Calif.	1934	10	0

The map (fig. 1) indicates that the "sex ratio" gene has a wide distribution in both races. In the case of race A it has a maximum frequency near the Mexican border, and decreases to the north, disappearing not far from the latitude of the northern boundary of California. The detailed data suggest also that the frequency decreases with increase in altitude. In southern California the Big Bear locality (7,000 feet) is much higher than the others (all under 4,000 feet), and has a lower frequency (1/17 as opposed to 11/47). Zion Canyon, Utah (where 4 out of 8 tested chromosomes carried sex ratio) is much lower than the neighboring localities (Cedar City, Bryce National Park, Grand Canyon) from which 17 tested chromosomes were all normal. In both of these cases the tests of wild males agree with those from sons of wild females.

The distribution of "sex ratio" in race B is less clear. There may be an area of maximum frequency on the coast near the Oregon-California line; but more data from other regions are needed.

PROPERTIES OF "SEX RATIO"

Table 3 shows the results obtained from a representative series of "sex ratio" males. It will be observed that the number of males per 100 females ranges from 0 to 17, with an average of about 3 in race A, 8 in race B. The exact numbers are probably not significant, since there are indications that the ratio may be modified by external agents such as tempera-

TABLE 3

Tests of "sex ratio" males (no sex-linked mutants present to produce viability complications)

RACE A				RACE B			
LOCALITY	♀♀	♂♂	♂♂ PER 100 ♀♀	LOCALITY	♀♀	♂♂	♂♂ PER 100 ♀♀
Wild males				*Sons of wild females*			
Zion Canyon	273	0	0	Gold Beach	873	66	8
Henshaw Lake	319	0	0	Oregon Caves	1063	29	3
Gila, near Yuma	303	2	1	Klamath	2584	262	10
Sons of wild females				Eureka	916	54	6
Pikes Peak	297	5	2	Shelter Cove	1334	110	8
Zuni	188	6	3	Lassen	657	74	11
Magdalena	304	4	1	Sequoia	529	58	11
Carizozo	116	20	17				
Pinos Altos	331	4	1				
Santa Catalina	374	26	7				
Flagstaff	460	0	0				
Chiricahua	528	5	1				
Santa Rita	379	6	2				
Zion Canyon	1101	10	1				
Santa Lucia	392	13	3				
Pasadena	239	9	4				
Durango	393	1	0				
Cuernavaca	743	0	0				

ture, and also that it may be influenced by modifying genes. As will be seen from the table, however, there is never any difficulty in determining whether a given male is giving the usual ratio of approximately 100 males per 100 females or the "sex ratio" result.

The sex ratios given by females carrying the gene in question (either homozygous or heterozygous) depend on the male used, and are not different from those given by homozygous normal females. It does not seem necessary to present numerical data to establish this relation.

Preliminary experiments soon established that the "sex ratio" gene is sex-linked, and attempts were then made to locate it with reference to the known sex-linked genes of *pseudoobscura*. Here an unexpected result was obtained: females heterozygous for "sex ratio" were found to give greatly reduced crossing over in the right limb of the X. This result has been obtained with "sex ratio" from several different localities (Santa Lucia, Pasadena, Chiricahua, Pikes Peak, Pinos Altos, Magdalena, Durango in race A; Santa Lucia, Sequoia in race B), and it has been shown (by testing crossovers) that the reduction in crossing over is definitely associated with

TABLE 4

Frequency of crossing over in various crosses involving the standard and the "sex ratio" in race A and B. In each case the frequency of crossing over is shown in percentages, and below is shown the total number of flies on which this percentage is based.

LOCI CROSS	*sr-y*	*sr-w*	*sr-f*	*y-v*	*y-co*	*w-s*	*v-f*	*v-s*	*f-mg*	*f-s*	*mg-s*	*co-s*
A+/A+				12.6 3617			4.0 1616		20.0 643		47.0 251	
A+/A *sr*				13.3 1732			2.1 1571		4.5 920		0.5 920	
A+/B+	0.0 109			8.7 492	23.8 235			25.4 492				4.7 235
A+/B *sr*	0.1 882				28.2 882							48.2 882
A *sr*/A *sr*				16.5 176				43.2 176				
A *sr*/B+		7.4 68				6.8 216						
B+/B+			10.0 1109							47.1 1109		
B+/B *sr*			13.7 1170							4.4 1170		

"sex ratio." Genetic studies of homozygous Pasadena "sex ratio" and cytological studies (in cells of the larval salivary glands) of Santa Lucia and Zion "sex ratio" heterozygotes agree in demonstrating that this reduction is due to the association of "sex ratio" with an inversion of a section of the right limb of the X chromosome, lying not far from the free end of the chromosome. The length of the inversion is equal to approximately one-fifth of the length of this limb, as observed in the salivary gland chromosomes. The only other crossover reducer met with in this chromosome limb genetically, and the only other inversion seen in it cytologically, is that in which races A and B differ from each other (LANCEFIELD 1929, TAN 1935). Accordingly it was desirable to see if race B "sex ratio" is also associated with an inversion, and also how the *sr* sequence of race A is related to the normal race B sequence. This problem has not been studied cytologically; but the genetic data of table 4 show the answer. There are three different sequences: the standard A which is identical with *sr* B; *sr* A; and standard B.

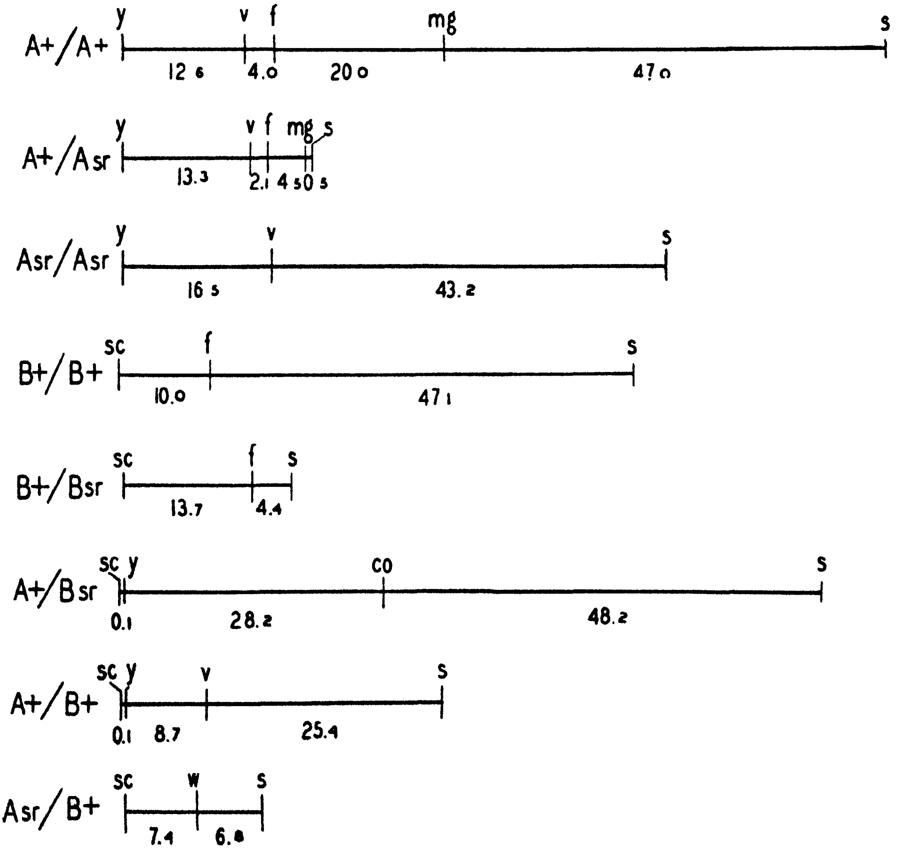


FIGURE 2. Crossing-over frequencies in the X chromosomes in various crosses.

In the case of standard A/*sr* B the actual data obtained are shown in table 5. Contrary classes are not equal in this case, a circumstance that is usual in A-B crosses (DOBZHANSKY and STURTEVANT 1935). However, it is clear that crossovers in both regions 2 and 3 (the latter including the "sex ratio" gene) are viable. None of the classes showed unusual characteristics in excess of the high variability usual in the offspring of A-B backcrosses, so it may safely be assumed that no net duplications or deficiencies were produced, that is, the sequence of genes in the two chromosomes was identical. In general the A-B hybrids show rather more crossing over than is to be expected by comparison with pure A or pure B. This is due to the effects of inversions in other chromosome limbs (SCHULTZ and REDFIELD, in MORGAN, BRIDGES, and SCHULTZ 1932).

TABLE 5

Crossing-over in $\dfrac{(\mathrm{A}+)\qquad y\quad co\quad s}{(\mathrm{B}\ sr)\ sc\ (1)\quad (2)\quad (3)}$ females

(+^{sc} classes in left-hand column; male offspring only)

Non-crossovers	81	245
Singles, Interval 1	1	0
Singles, Interval 2	93	37
Singles, Interval 3	122	184
Doubles, 2 and 3	65	54

TESTS WITH II-Y TRANSLOCATION

SCHULTZ (1933) has reported briefly on a dominant mutant type known as Delta, that was associated with a II-Y translocation. Delta males, mated by normal females, gave mostly normal daughters and Delta sons, plus a few Delta daughters (due to the production of XY sperm) and a few normal sons (XO, due to the production of sperm with neither X nor Y). This translocation was tried against "sex ratio" (Pasadena, A). From normal ♀ X*sr*/Delta ♂ there were produced 1907+ ♀, 166+ ♂, 14 Delta ♂. This result was unexpected, and remained unexplained until the cytological study described below. It is now clear that it was correct in indicating that the Y chromosome is actually absent in most of the sperm of a "sex ratio" male. In the present case the absence of Delta females is even more marked than in the case of +/Delta male, where about 2.6 percent of the daughters were Delta; by comparison at least 49 should have been expected here, whereas none was found.

SPERMATOGENESIS IN "SEX RATIO" MALES

As shown above, the offspring of a male carrying "sex ratio" come from eggs fertilized by spermatozoa possessing an X chromosome but no Y chromosome. Only a small fraction of the eggs are fertilized by Y-bearing sperm, or by spermatozoa free from either X or Y chromosomes. Since no significant increase of zygotic mortality is observed in sex ratio cultures, the possibility that the eggs fertilized by Y-bearing spermatozoa are inviable is excluded. Hence, the explanation of the behavior of the "sex ratio" may be sought along one of the following two lines. First, the X-bearing and the Y-bearing spermatozoa may be produced in equal numbers, but a majority of the latter may for some reason fail to fertilize the eggs. Second, the spermatogenesis may be so modified that only X-bearing sperm are produced. A cytological study was undertaken with the aim of securing some evidence bearing on this problem. A strain of race A rather than of race B, was selected for this purpose, because in the former the X and Y chromosomes are clearly distinct from each other in meiotic stages, whereas in race B the two chromosomes are more similar.

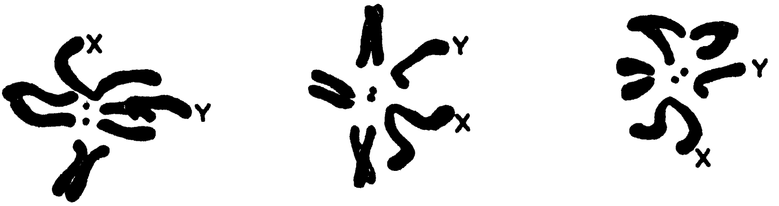


FIGURE 3. Spermatogonial metaphase plates from males carrying "sex ratio." X = X chromosome, Y = Y chromosome.

Males from one of the "sex ratio" strains were crossed to temales homozygous for the sex-linked recessives eosin, magenta, and short. The F_1 females from cultures showing abnormal sex ratios were crossed to normal males, and in the next generation non-magenta males were selected. Since "sex ratio" is closely linked with magenta, these males must carry it. Testes of young males were fixed in Benda's or Navashin's fluid, sectioned 7μ thick, and stained in iron haematoxylin. In the following description the spermatogenesis of the "sex ratio" males is compared with that in normal ones. For the information concerning the latter see the papers by DARLINGTON (1934) and DOBZHANSKY (1934).

The size, shape, and general structure of the testis in "sex ratio" males is normal. The spermatogonia are likewise normal; their resting nuclei have one, less frequently two, nucleoli with one or two satellites. The spermatogonial divisions (fig. 3) show a V-shaped X and a J-shaped Y chromosome, the somatic pairing being apparently as strong as in normal spermatogonia.

The first spermatocytes (fig. 4a) have nuclei with the usual reticulum and several nucleoli of various sizes. Especially in preparations fixed in Navashin's fluid, a careful examination shows that one of these nucleoli, usually the largest one, is really a precociously condensed chromosome. It is split equationally, and appears as a very short and stout V, or as a broken ring, or as an angular mass. In strongly destained preparations this chromatin nucleolus, which is the Y chromosome, retains the stain longer than other nucleoli (fig. 4a). Since a precocious condensation of the Y chromosome has not been noticed in the normal spermatogenesis, a reexamination of the slides of normal testes has been made. The normal first spermatocytes have from one to several nucleoli; the single, or the largest of the several, appears usually as a regular sphere. In strongly destained or faded preparations the sphere shows a lighter core and a dark surface. Only seldom faint indications of doubleness in the nucleolus are noticeable. The relations between this nucleolus and the Y chromosome are uncertain. (Dr. Hans Bauer kindly informs us that in his preparations of the normal testes stained by the Feulgen method the nuclei of the first spermatocytes show no chromatin nucleolus. No satisfactory preparations of "sex ratio" testes stained in Feulgen's have been seen, but here the relation between the nucleolus and the Y chromosome is rather clear. It follows that the precocious condensation of the Y chromosome in the nuclei of the first spermatocytes is the first visible sign of abnormality in the spermatogenesis of "sex ratio" males.)

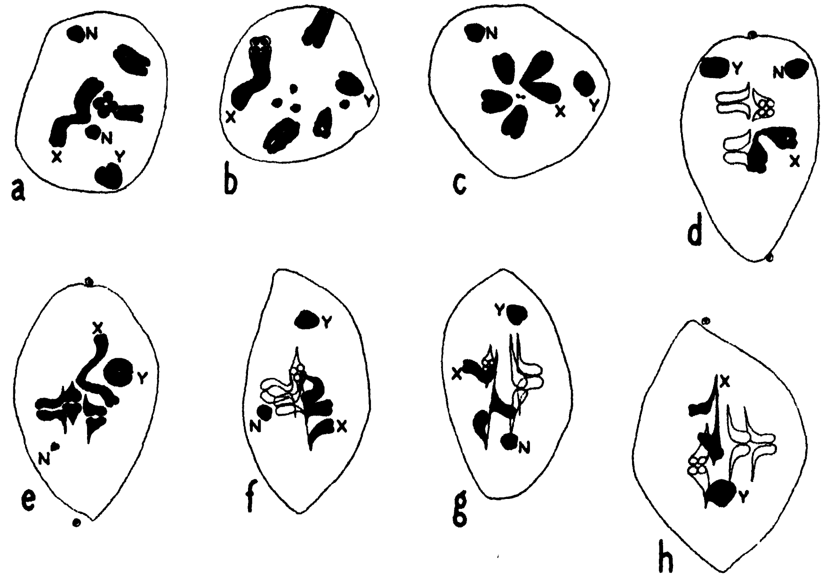


Figure 4 Spermatogenesis in "sex ratio" males. a, b—diakinesis; c–h—metaphase and anaphase of the first meiotic division. N—nucleolar fragments; X—X chromosome; Y—Y chromosome. In d, f, g, and h only outlines of the autosomal bivalents are represented.

At diakinesis (figs. 4d, 5a, b) three autosomal bivalents and unpaired X and Y chromosomes become visible. The autosomal bivalents appear in side view as two rather intimately paired rods, and in end view as four equidistant dots. The appearance and the behavior of the autosomal bivalents at diakinesis and the first meiotic division are normal. The Y chromosome is strongly contracted, but the equational split is usually clearly visible (Y, figs. 5a, b). In early diakinesis (fig. 4d) the autosomes and the X chromosome appear, as usual, first as pale brownish bodies which acquire the full stainability only gradually. The Y chromosome stains much darker; its origin from the "chromatin nucleolus" of the preceding stage can be followed with certainty. The X chromosome, despite being an apparent univalent, is uncommonly large and stout (figs. 5a–d). In favorable cells, in which one or both ends of the X chromosome are directed toward the observer, the cause of this is visible: this chromosome has two equational splits instead of the normal one, and is therefore quadripartite instead of bipartite. Whether this quadripartite structure is present in all cells is doubtful, since in some the X chromosome is rather slender (figs. 4d, 5e), but it may be taken for certain that most cells do have quadripartite X's. It is, however, impossible to determine by direct observation how far the double equational split goes in the body of the chromosome (as just stated, it is visible in the ends only). Since in normal

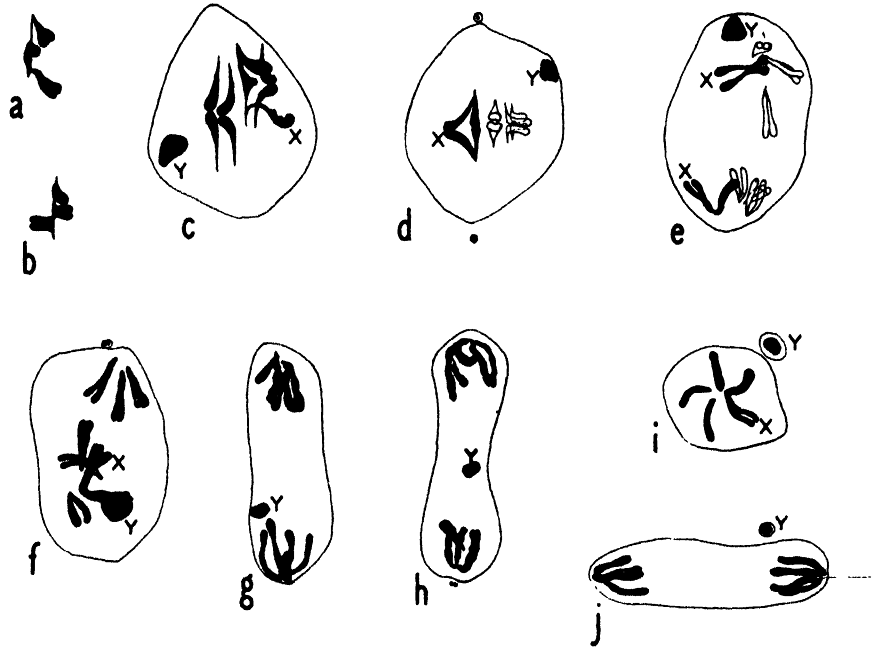
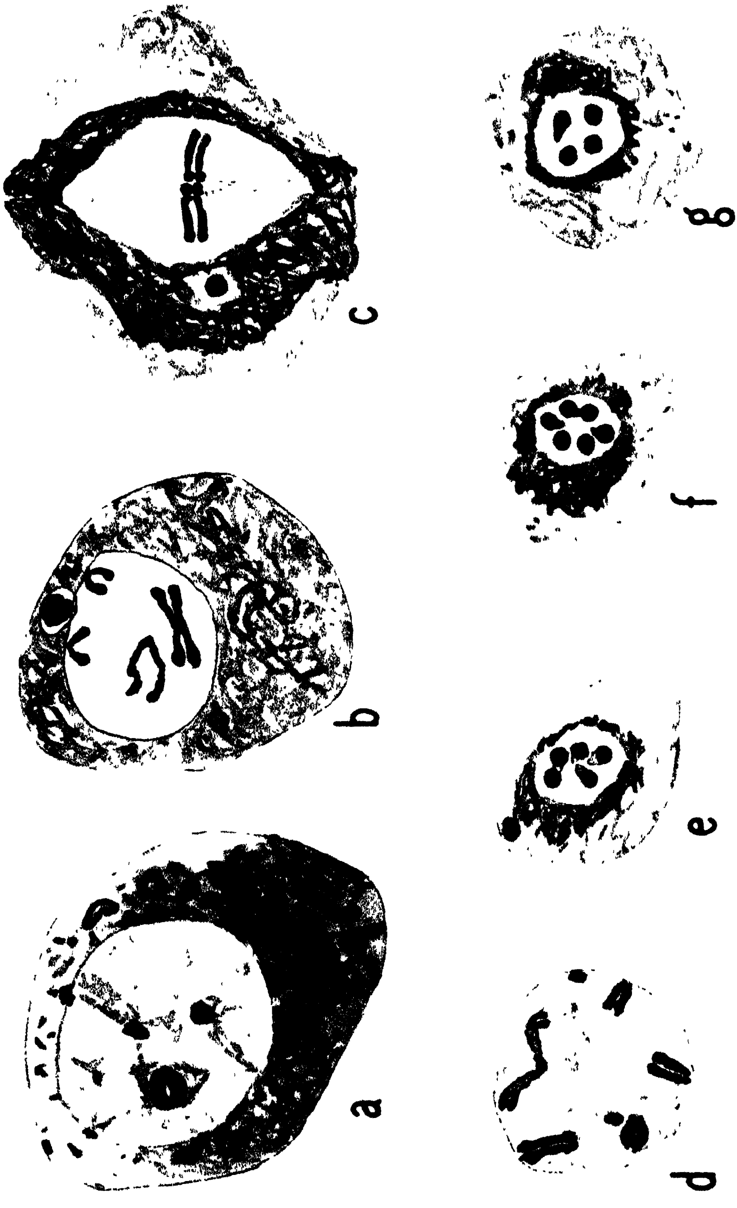


FIGURE 5. Spermatogenesis in "sex ratio" males. a and b—the X chromosome at anaphase; c and d—anaphase of the first meiotic division; e–h—telophase of the same; i—metaphase, and j—telophase of the second meiotic division. X and Y— the X the Y chromosomes respectively.

gametogenesis each chromosome is split into two sister strands along its entire length except at the spindle attachment where the two strands are held together, it seems simplest to assume that here too we have four chromatids converging to an undivided spindle attachment. Such an interpretation is in agreement with the observations on the later stages.

At metaphase the autosomes and the X chromosome become arranged in the loose equatorial plate characteristic for the first meiotic division in *Drosophila pseudoobscura* (figs. 5c–h). The Y chromosome takes no part in the equatorial plate and may lie anywhere on the spindle, in the equatorial region as well as in the polar ones. The extraordinary persistence of the nucleolar fragments (N, figs. 5a–g) during the first division seems to be characteristic for the spermatogenesis in the "sex ratio" males. In normal spermatogenesis the nucleolus of the first spermatocytes becomes pale and disintegrates already during the mid-diakinesis, although in some cases the fragments are visible up to metaphase. In the "sex ratio" the fragments are seen even in early anaphase (figs. 5f, g), and some of them are so large and heavily staining that for a time it was suspected that the univalent Y chromosome divides in some cells as early as the first division, which proved to be an error.

At anaphase (fig. 4f–h, fig. 5a–c) the spindle attachment in the quadripartite X chromosome evidently undergoes a division, for the two daughter chromosomes, each showing one equational split, become directed toward opposite poles and away from each other. However, no immediate and complete separation takes place. One of the two arms of each daughter chromosome becomes free, while the other arms are held together for some time. The resulting configurations have a striking resemblance to those observed in the X-Y bivalents of normal *Drosophila pseudoobscura* by DARLINGTON (1934, fig. 20), and attributed by him to the presence of two reciprocal chiasmata in one of the two arms of each chromosome. Whether or not the association of the two X chromosomes in our case is also due to chiasma formation is uncertain. An alternative explanation would be that the second equational split takes place in one arm somewhat later than in the other, and that the unsplit arms are temporarily held together by some force, for instance by an exaggerated somatic pairing. This involves the assumption that the division of chromonemata occurs in a condensed metaphase chromosome. One must also take into account that in some cells, more or less exceptional ones, somewhat different configurations may be seen, as in figure 5d. Here both arms are associated with their homologues at their ends; one of the arms seems single rather than double, although this is not certain. In a few cells (fig. 5f) the division of the spindle attachment does not occur until late anaphase, and the quadripartite X chromosome lags on the spindle.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f
g

At late anaphase and early telophase (fig. 5e, g, h) three autosomal diads and one X chromosome may be seen passing to each pole. The X is split once equationally, and resembles in all particulars the X seen at one of the two poles in normal spermatocytes. The Y still continues to lag in the spindle; it remains much condensed and its spindle attachment end is not attenuated. The next stage is the division of the cell and the formation of second spermatocytes

The second spermatocytes (Plate 1b) are normal except for the fact that in some of them besides the normal nucleus also a small micronucleus is found. The micronucleus contains a single chromosome which is evidently the Y chromosome. It follows that during the first meiotic division the Y is not included in either of the two telophase groups, but is left behind, and at the time of the fission of the cell body gets into one of the daughter cells forming there a separate small nucleus. The chromosomes in the second spermatocytes can frequently be counted; all among the sixty-four spermatocytes examined had an X chromosome and no Y chromosomes in their main nuclei It should be remembered, however, that the "sex ratio" strain used by us for the cytological investigation produces practically only females, and that other strains give rise to some males as well. What happens in the latter is unknown, it is possible that cells like that shown in fig 4e give rise to one second spermatocyte containing the X and one containing the Y. It is also possible that normal X-Y bivalents occur at the first division in some cells in "sex ratio" males. Some Y-bearing spermatids were found in our strain as well (see below).

The second meiotic division (Plate 1c, figs. 5i, j) is perfectly normal. The chromosomes in the main nucleus form an equatorial plate, and at anaphase the equational halves pass to the opposite poles. The micronuclei containing the Y chromosome take no part in this division. They are not included in the spindle, nor do they form small spindles of their own. The chromosome in the micronucleus becomes small and exceedingly contracted; the equational split in it is no longer visible. It is included in one of the resulting spermatids. At the telophase of the second division the chromosomes in the polar groups can be counted if the spindle is viewed from the pole (Plate 1c g). Most cells show five dots (Plate 1e), and by focusing it is possible to see that two of the five dots unite at a lower level. These are the two limbs of the X, hence one X and three autosomes are present in such a telophase group. Such counts were made in 200 telo-

DESCRIPTION OF PLATE I

Spermatogenesis in "sex ratio" males. a—first spermatocyte; b—a second spermatocyte with a micronucleus; c—the second meiotic division; d - early diakinesis, e, f, and g polar views of the telophase groups at the end of the second meiotic division.

phase groups. The results were: 195 groups with X but no Y; 3 groups with a Y but no X (Plate 1g); and 2 groups with one X and one Y (Plate 1 4f.)[1]

The spermatogenesis is normal: some of the young spermatids have a micronucleus with the Y chromosome, but in somewhat later stages the micronuclei are no longer visible. What is their final fate is uncertain; probably they are discarded together with the excess cytoplasm. Cross-sections of the cysts of mature or semi-mature spermatozoa offer the opportunity to count the number of spermatozoa per cyst. The normal number is close to 128 (meiosis occurs in groups of 32 cells, this results in 128 spermatids, DOBZHANSKY 1934). In the "sex ratio" males about four cysts were counted, and the resulting numbers were not far from 128. There is no indication whatever that any spermatids die, or that some of the spermatozoa in a cyst are abnormal.

OCCURRENCE OF "SEX RATIO" IN OTHER SPECIES

As pointed out in the introduction to this paper, "sex ratio" has been reported in *D. affinis* Sturtevant and *D. obscura* Fallén. These two species are rather similar to *D. pseudoobscura* Frolowa; there are also several European and North American species of the same group (mostly undescribed), some of which we have investigated. The European species are being studied by Mr. J. E. COLLIN (Newmarket, England), whose manuscript name *subobscura* is here used for the commonest British species. This is the form whose chromosomes are listed (*fide* C. W. METZ) by MORGAN, BRIDGES and STURTEVANT (1925, p. 182), under the designation "undescribed European species near *D. obscura*." The species here listed as *obscura* Fallén may or may not be the same as that used by GERSHENSON (1928), since the cytological account of FROLOWA and ASTAUROW (1929) indicates that at least two Russian forms occur, both different from METZ's account of *subobscura*. We have in preparation an account of some of the American forms; the species here listed as *athabasca*, *azteca*, and *algonquin* will be described there.

Table 6 shows the data obtained from wild specimens of these various species—data of the same type as those of tables 1 and 2 show for *D. pseudoobscura*.

In the cases of *affinis* and *athabasca* from Woods Hole, and of *azteca* from Cerro San Jose, Oaxaca, Mexico, wild females were found that gave few or no sons. We may assume that these had mated with "sex ratio" males.

"Sex ratio" thus occurs in *pseudoobscura* A, *pseudoobscura* B, *obscura*, *affinis*, *athabasca*, and *azteca;* it was not found in *subobscura* or *algonquin*, but may well be present in them in other localities. The question arises, are we dealing with the same gene in all these cases? That the essential properties are the same is clearly indicated. As shown above, in both races of *pseudoobscura* the gene is located in the right limb of X, is associated with an inversion, and males carrying it give similar frequencies of sons. In *obscura*, GERSHENSON has established all these points except the region occupied in the X and the presence of an inversion. In *affinis* we have found a similar frequency of sons, have shown the gene to be in the X, and salivary gland preparations of heterozygous females show an inversion in the X. In *athabasca* and *azteca* the evidence is less complete, resting chiefly on the sex-ratio itself, though some experiments with *athabasca* are at least consistent with the sex-linkage of the gene concerned.

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[1] **This figure may also be interpreted as resulting from non-disjunction of one of the autosomes; if so an X, four autosomes, and no Y are visible in this group.**

TABLE 6

Occurrence of "sex ratio" genes in species other than D. pseudoobscura

SPECIES	LOCALITY	YEAR	X'S OF WILD ♀♀		WILD ♂♂	
			+	sr	+	sr
obscura	Berlin, Germany	1933	2	0	1	0
obscura	Birmingham, England	1932	2	0	3	0
obscura	Newcastle, England	1933	0	0	5	0
obscura	*Moscow, Russia (GERSHENSON 1925)	1925	36	2	0	0
subobscura	Berlin, Germany	1933	0	0	3	0
subobscura	Birmingham, England	1932	17	0	77	0
subobscura	Newcastle, England	1933	19	0	26	0
affinis	Woods Hole, Mass	1932	4	0	12	1
affinis	Woods Hole, Mass.	1933	3	0	0	0
affinis	Woods Hole, Mass.	1935	12	2	95	8
affinis	Kushla, Ala.	1935	7	0	8	0
algonquin	Woods Hole, Mass.	1932	1	0	1	0
algonquin	Woods Hole, Mass.	1933	2	0	0	0
algonquin	Woods Hole, Mass.	1935	8	0	10	0
algonquin	Mendham, N. J.	1932	2	0	0	0
athabasca	*Woods Hole, Mass.	1935	6	1	1	2
athabasca	Quesnel, B. C.	1934	3	0	0	0
athabasca	Kaslo, B. C.	1934	4	0	0	0
athabasca	Shuswap Lake, B. C.	1934	2	0	0	0
athabasca	Arrowhead, B. C.	1934	2	0	0	0
athabasca	Cape Flattery, Wash.	1934	2	0	0	0
azteca	Cuernavaca, Mexico	1935	5	0	0	0
azteca	Oaxaca, Mexico	1935	2	0	0	0

* Species identification somewhat uncertain.

DISCUSSION

Some items in the spermatogenesis of the "sex ratio" males have a rather general interest. In the first spermatocytes the X chromosome undergoes two equational splits while the rest of the chromosomes split once. This shows that the division of the chromonemata is not necessarily induced by the physiological condition of the nucleus as a whole, but can proceed independently in different chromosomes. This is not new, for in some interspecific hybrids the univalent chromosomes may undergo splitting both at the first and at the second meiotic divisions, while the bivalents split equationally only once. Nevertheless, our case remains unique since here the extra split in the chromosome is known to depend upon the presence of a factor (or a group of factors) localized in a relatively short section of the chromosome involved. The further behavior of the quadripartite X chromosome, and especially the formation of the chiasma-like association between the division products in this chromosome, clearly has a bearing on a number of theoretical problems connected with crossing-over and general chromosome structure. Since, however, we do not consider it established that real chiasmata are here found, a further discussion of this point is premature.

Following DARLINGTON, one might suppose that the reason for non-pairing of the X and Y chromosomes in the "sex ratio" gametogenesis is the extra split in the X chromosome. This, however, helps little in accounting for the subsequent behavior of the X and Y chromosomes. Why, for instance, does the Y chromosome show a precocious condensation and heteropycnosis, and the X behave like the autosomes? Or why is the Y chromosome left in the cytoplasm instead of becoming included in one of the telophase groups? Its univalent condition does not account for either of these phenomena, since the univalents in the hybrids between the A and B races of *Drosophila pseudoobscura* are frequently included in the telophase groups, and if they form separate micronuclei they show signs of activity at the next division (DOBZHANSKY 1934). The only visible difference between the univalents in the hybrids and the univalent Y in the "sex ratio" is that in the former the spindle attachment becomes active after a more or less prolonged delay (as shown by the attenuation of the attachment region), while in the latter no such "activity" is observed. The inherent difficulty of the causal analysis of the behavior of the Y chromosome in the "sex ratio" males lies in the fact that the genetic factor responsible for the whole complex of the deviations in the course of the spermatogenesis is located in the X chromosome only. Despite this, much of the abnormality concerns not the X but the Y chromosome.

"Sex ratio" is associated with an inversion (as compared with the normal X) in both races of *pseudoobscura* and in *affinis* (*obscura*, *athabasca*, and *azteca* have not been examined for this point). It seems clear that this association cannot be an accidental one, but there are two reasons for concluding that the inversion as such is not an essential part of the mechanism responsible for the peculiarities of the "sex ratio" X. First, these peculiarities occur only in males which have a single X and are not heterozygous for an inversion, since the region of the X concerned is presumably not one that is in any sense homologous to the Y. Second and more significant, the "sex ratio" sequence of *pseudoobscura* race B is identical with the normal sequence of race A, so that "position effects" resulting from the inversion are excluded. One possible interpretation of the significance of the inversions is suggested below.

GERSHENSON (1928) pointed out that "sex ratio" should automatically increase in frequency in any population containing it, since a heterozygous male transmits the gene to nearly all his offspring, while a female (either homozygous or heterozygous) transmits it in the same frequency as any other gene. Clearly this would be fatal to the race if it did occur; we have, for example, collected a fertilized wild female that gave 236 daughters and one sterile son. It is equally clear that the expected increase in frequency does not occur in nature. Wild populations are somehow kept in equilibrium; but the nature of the counteracting influence can only be surmised. It must be of such a magnitude that it brings about a result equivalent to the production of only about half as many offspring by a "sex ratio" male as by a normal one, on the average. Such an influence should be easy to detect experimentally, but preliminary attempts to locate it have not been successful. These studies are being continued.

GERSHENSON'S point suggests a possible interpretation of the significance of the inversions associated with "sex ratio." If "sex ratio" is in reality not one gene, but two or more complementary ones located in the same general region of the X, then in the absence of an inversion the two would constantly be separated by crossing over and the automatic increase in frequency would be slow. If an inversion occurred in an X that happened to carry both of these hypothetical genes, their separation would be prevented and automatic increase would set in.

The algebraic analysis of populations containing "sex ratio" is difficult, and may best be postponed until more evidence is available concerning the nature of the equilibrium that occurs. These remarks apply even more obviously to the hypothesis of complementary genes just suggested.

SUMMARY

1. "Sex ratio" (symbol *sr*) lies in the right limb of the X of races A and B of *D. pseudoobscura*.

2. Males carrying *sr* give offspring consisting mostly of females, regardless of the nature of their mates.

3. Cytological study shows that, in "sex ratio" males, the X undergoes an equational division at each meiotic division, the Y degenerates, and the autosomes behave normally.

4. The *sr* gene is widely distributed in wild populations of both races of *pseudoobscura*, and what appears to be the same gene occurs also in wild populations of the closely related species *obscura*, *affinis*, *athabasca*, and *azteca*.

5. "Sex ratio" is associated with an inversion in both races of *pseudoobscura* and in *affinis*, the other three species not having been studied.

6. In *pseudoobscura* three sequences occur: *sr* A, normal B, and one that is common to normal A and *sr* B.

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A CYTOGENETIC STUDY OF A CHROMOSOME FRAGMENT IN MAIZE[1]

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INTRODUCTION

EACH of the ten groups of linked genes in maize has been associated with a specific member of the haploid set of ten chromosomes. The morphology of the ten chromsomes has been studied at pachytene by McCLINTOCK (1933) and each member found to differ from the others in such characters as length, position of the spindle fiber insertion region and presence of deeply staining knobs in definite positions, so that they can be recognized by their morphological characteristics.

The fifth longest chromosome of the haploid set of maize has a spindle fiber insertion region that is nearly median, dividing the chromosome into two arms. The ratio of the length of the two arms is 1.1:1.0. In certain strains the longer of the two arms, however, is differentiated at pachytene by the presence of a deeply staining knob that makes it easy to distinguish between the two nearly equal arms of the chromosome.

Plants which possess an extra chromosome V are said to be trisomic for this chromosome. These trisomic plants differ markedly in appearance from their disomic sibs and it is possible to classify a segregating progeny into trisomic and disomic types with considerable accuracy. For this reason plants trisomic for chromosome V have been successfully utilized in an attack on certain problems.

There are two secondary trisomes possible for each primary trisome since the extra chromosome in the secondary trisome is composed of two identical arms, that is, one of the arms of the chromosome is represented twice in the extra chromosome. One of the two secondary trisomes for chromosome V has been found (RHOADES 1933b). The extra chromosome consisted of two short arms of chromosome V. This secondary differed strikingly in appearance from both primary trisomes and disomes.

OCCURRENCE OF A FRAGMENT PLANT

An individual which was intermediate in its appearance between its trisomic and disomic sibs arose in the progeny of a chromosome V primary trisome. Cytological examination of this exceptional plant showed that it contained a fragment of chromosome V. Studies at pachytene showed that this fragment consisted of the entire short arm of chromosome V with a terminal spindle fiber insertion region. That the break which produced the fragment occurred exactly at the spindle fiber region is indicated by the fact that in many clear pachytene figures no evidence of any chromatic material on one side of the insertion region was ever found. The nature of this fragment in its relation to a normal chromosome V is shown diagrammatically in figure 1.

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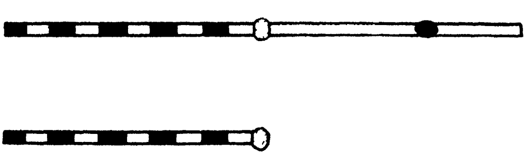

FIGURE 1.—Diagrammatic representations of normal chromosome V showing relative length of the two arms, position of spindle fiber insertion region, location of knob and of fragment chromosome which consists of the entire short arm of chromosome V and which has a terminal spindle fiber insertion region The insertion regions are designated by the stippled areas.

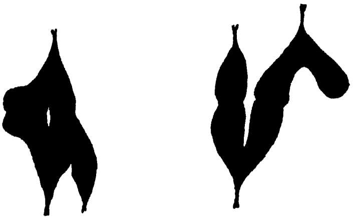

FIGURE 2. Trivalent groups of the two normal and the fragment chromosomes at metaphase I showing the orientation of the fragment chromosome to be such that it disjoins to the same pole as one of the normal chromosomes

As previously reported, maize plants trisomic for chromosome V differ markedly in their appearance from their disomic sibs. Especially noticeable are the thick broad leaves with relatively blunt tips, the stubby tassel and the reduced height of the trisomic plants. Plants carrying the short arm of chromosome V in excess are intermediate in appearance between primary trisomes and disomes.

CYTOGENETICS OF FRAGMENT CHROMOSOME

Cytological observations of microsporocytes at metaphase I showed that the fragment chromosome was associated with the two normal chromosomes V in approximately 53 percent of the cases to form a trivalent group. This frequency of trivalents is considerably lower than the 85–90 percent found in plants trisomic for the whole chromosome V. If assortment of the members of the trivalent group is completely random six gametic types are possible. If these six types are produced with equal frequencies the genetic backcross ratio from a plant carrying two dominant and one recessive alleles (*AAa*) would be 5*A*:1*a*. (This ratio ignores the effect of chromatid crossing over on genetic ratios.) Random assortment of the three homologous chromosomes occurs in primary trisomes but the occurrence of univalents and their failure to pass to either pole in anaphase results in a deviation of the genetic ratio from a 5:1 to about 3.35:1 (RHOADES 1933a) for genes in chromosome V.

Typical trivalent figures involving the fragment chromosome are shown in figure 2. The orientation on the metaphase plate of the trivalent group was such that the fragment would pass to the same pole as one of the normal chromosomes and was rarely of the type that would permit the two normal chromosomes to pass together to one pole while the fragment went to the other pole. Only four gametic types are usually formed. The theoretical gametic ratio for genes located in the short arm would be $3A:1a$ if the four gametic types are formed with equal frequencies and if the dominant allele is carried in the fragment chromosome and in one of the two normal chromosomes.

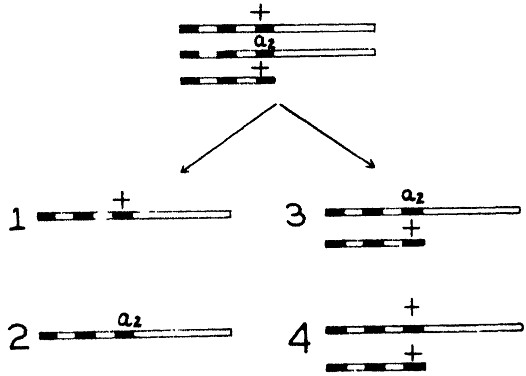

FIGURE 3.—Diagrammatic representation of the four gametic types produced by a fragment plant. The locus a_2, which is included in the fragment, is used to mark the different chromosomes.

The rare occurrence of primary trisomes in the progeny from fragment plants substantiates this idea of a non-random segregation of the members of the trivalent group.[3] Since the spindle fiber insertion region of the fragment chromosome is comparable in size with that of the two normal chromosomes, the failure of occurrence of random segregation indicates that the insertion region does not of itself control chromosome segregation. Such a conclusion has been reached on genetic grounds by DOBZHANSKY and STURTEVANT (1931) in their studies on Drosophila translocations. The four gametic types from a fragment plant are shown diagrammatically in figure 3.

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[3] Actually 1.5 percent of the progeny from a fragment plant, used as the female parent, are primary trisomes. The occurrence of these primary trisomes has a slight effect upon the genetic ratios but this has been disregarded to facilitate calculations.

When a trivalent group was present at metaphase, disjunction occurred and the three chromosomes were distributed between the two poles. If a bivalent and a univalent were present, however, the behavior of the univalent was variable. Often it never reached the metaphase plate and remained in the cytoplasm during the formation of the daughter nuclei. If it reached the metaphase plate it was often late in beginning its migration to one of the two poles and consequently was not included in the telophase nuclei. Occasionally the univalent apparently succeeded in reaching one of the poles and was, therefore, included in the nucleus.[4] So far as observed the univalent never divided in the first meiotic division. If a univalent was present it was always the fragment chromosome.

Knowing that only four gametic types are formed, that trivalent groups are formed at metaphase in about 50 percent of the cells, we can calculate the gametic ratio for a gene located in the short arm of chromosome V if we assume that only those fragment chromosomes which were members of trivalent groups reach the pole. Based on the above the expected back-cross ratio from fragment plants of AAa constitution with a in one of the normal chromosomes is $5A:3a$ or 37.5 percent recessives.

Studies at pachytene showed that the fragment was often paired with the two normal chromosomes. The fragment was paired in approximately one-half of the microsporocytes. The difficulty in interpreting all figures makes this ratio of doubtful value but there was at least rough agreement between the frequency of trivalent complexes observed at pachytene and at metaphase.

That the insertion region is not the place where synapsis must be initiated is indicated by the following observations at pachytene. In those microsporocytes in which the fragment had paired with the short arms of the two normal chromosomes V the terminal insertion region of the fragment chromosome was usually some distance removed from the insertion regions of the normal chromosome. In most synaptic configurations it was some distance from the terminal insertion region of the fragment to the place where the fragment first paired with one of the normal chromosomes. Pairing between the three homologous arms was always between two of the three chromsomes, that is, one of the chromosomes was always unpaired at any given region. There was never any tendency for all three chromosomes to be associated at the same point. The fact that the insertion region of the fragment chromosome had no tendency to be associated with the insertion regions of the normal chromosomes V indicates that the insertion regions in themselves have no specific attraction. In disomes at pachytene the insertion regions of two synapsed chromosomes lie side by side. This is probably caused by their being brought together by the synapsing of the homologous loci on either side and not because of any specific attraction to one another. It was not uncommonly observed that even though the three homologous arms were involved in a synaptic complex the terminal insertion region of the fragment was attached or stuck to the insertion regions of other pairs of chromosomes. This "stickiness" of insertion regions has often been observed in normal preparations and is not a characteristic feature of the insertion region of the fragment chromosome.

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[4] If the fragment chromosome was paired with the two normal chromosomes at metaphase it had a characteristic V-shaped appearance during anaphase I as it passed to one of the two poles. On the other hand if the fragment chromosome was unpaired at metaphase it had a ball-like or spherical shape at anaphase. In an occasional figure a spherical fragment chromosome accidentally lay so near one of the poles that it probably would be included in the telophase nucleus.

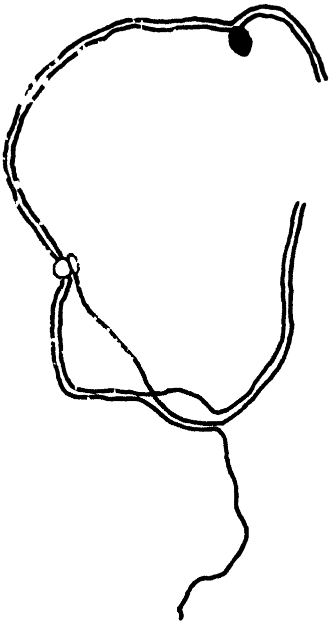


FIGURE 4.—Camera lucida sketch of synaptic relationships of the fragment chromosome and the two normal chromosomes V at pachytene. In this figure the insertion region of the fragment is in juxtaposition with the two insertion regions of the normal chromosomes. One of the normal chromosomes has a deeply staining knob in the long arm.

In those sporocytes in which the fragment was present as a univalent at pachytene it was less contracted than the paired short arms of the two normal chromosomes V with which it is homologous. HUSKINS and HEARNE (1933) observed a similar attenuation in unpaired chromosomes. Non-homologous pairings (foldbacks) were of infrequent occurrence in the unpaired fragment. The equational split of the unpaired fragment chromosome into two chromatids was clearly evident at pachytene. Of considerably more interest was the observation that the terminal insertion region of the unpaired fragment in some cells, where it was not stuck to other insertion regions, appeared to be split or divided at pachytene. The distal end of the insertion region was cleft to form a heart-shaped structure. As the diagrammatic sketch of the fragment chromosome in figure 5 illustrates there is a concentration of deeply staining chromatin near the insertion region while the more distal portions contain much less deeply staining material. A considerable amount of genetic data strongly suggests that the disjunction of the insertion regions is always reductional in the first meiotic division. DARLINGTON (1931) in his theory of meiosis accounts for this by assuming that the split of the insertion region occurs some time after the equational split of the chromosome into two chromatids. If the observation of the split nature of the terminal insertion region of the unpaired fragment is to be considered applicable to paired chromosomes, then the previous failure to observe the double nature of the insertion region at midprophase is probably due to its homogenous appearance and because its usual interstitial location is such as to conceal the split if it occurred. Only when the insertion region is favorably located, that is, terminally, could the split be evident. There is, of course, the possibility that the split condition of the insertion region of the unpaired fragment is not representative of conditions in paired chromosomes with internal insertion regions. Moreover, if the insertion does split at the same time as the chromosome there is no reason why the two daughter insertion regions cannot behave as "one effective insertion region" at metaphase and anaphase.

LEWITSKY (1931) and others hold that no chromosome has a strictly terminal insertion region. It is true that none of the normal complement of maize chromosomes (MCCLINTOCK 1933) has a terminal insertion region but LEWITSKY's statement does not hold for the fragment chromosomes described in this paper since conditions for cytological observations are exceptionally good at pachytene in maize and the fragment chromosome clearly had a terminal insertion region. The fragment chromosome was as stable as the regular complement of 20 chromosomes in its behavior throughout all the somatic mitoses and no irregularities were manifested.

LOCATION OF GENES IN LONG AND SHORT ARMS OF CHROMOSOME V

Since the fragment consists of the entire short arm of chromosome V it can be employed in determining which of the genes located in this chromosome are situated in the short arm and which are in the long arm. The A_2 locus will be used as an illustration of how this was accomplished. A plant carrying the fragment and homozygous for A_2 was pollinated by a plant homozygous for a_2 and for A_1CR, the other factors concerned with aleurone color. The F_1 fragment plants were then backcrossed with recessive a_2 pollen. If the A_2 locus is in the short arm of chromosome V a deviation from a 1:1 backcross ratio will be had for A_2:a_2. The magnitude of the deviation will depend upon the frequency with which the fragment chromosome, which carried the dominant allele, is included in functioning female gametes. The fragment chromosome was paired with the two normal chromosomes at metaphase I in about 50 percent of the microsporocytes. If we assume that a similar condition holds for the megasporocytes and if we make the further assumption that the unpaired fragment chromosome does not succeed in reaching either of the two poles in anaphase I, and is consequently lost, the expected ratio of A_2:a_2 seeds in the backcross of fragment plants is 5A_2:3a_2 seeds. Table I gives the ratio of A_2:a_2 seeds actually found and indicates that A_2 is in the short arm of chromosome V. Disomic sister plants gave 869 A_2 seeds to 890 a_2 seeds or a 1:1 ratio. The average of 33.6 percent recessives suggests that either approximately 67 percent of the megasporocytes had a trivalent group or else that the unpaired fragment reached one or the other of the two poles in the first anaphase with a considerable frequency. A more useful and efficient test for the presence of any locus in the short arm is the occurrence of fragment plants which are homozygous for the recessive gene in the backcross progeny of a fragment plant carrying one recessive allele in a normal chromosome. If the locus under consideration is in the short arm, no fragment plants will occur, barring the rare occurrence of exceptional individuals through chromatid crossing over, which are homozygous for the recessive allele. This latter test was used for all the genes tested, as it required fewer plants and was of a more critical nature.

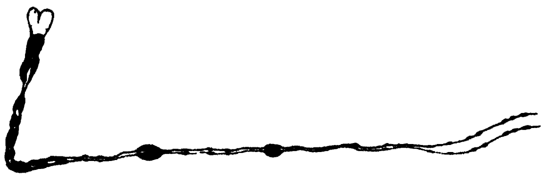


FIGURE 5. Diagrammatic sketch of an unpaired fragment chromosome at pachytene. The equational split into two chromatids has occurred and the heart-shaped appearance of the terminal insertion region suggests that it also is double at this stage. There is more deeply staining chromatin near the insertion region than in the more distal portions of the chromosome.

Linkage group 5 contains 23 genes. The order of the following genes in the genetic map is correct, $\frac{a_2\ bm_1\ bv\ pr\ ys\ v_2}{0\ \ 6\ \ 12\ \ 31\ \ 40\ \ 72}$, according to the data summarized by **Emerson** et al (1935). The exact location of the other genes in the linkage map is uncertain. Eight of the 23 genes in this chromosome were tested against the fragment. The V_2, Ys_1, Pr, V_{12}, V_3 and Bt_1 loci were found to be in the long arm of chromosome V while only A_2 and Bm_1 were situated in the short arm. Of especial interest is the placing of Bm_1 and Bt_1 on opposite sides of the spindle fiber insertion region as these two loci show little crossing over (BURNHAM and RHOADES in EMERSON et al 1935). A_2 is only some 6–7 crossover units from Bt_1, which is in the long arm, and it occupies, at present, the leftmost position of all the factors in the genetic map of chromosome V. If the magnitude of crossing over per unit of physical length is the same in the two arms of the chromosome, and we have no reason to believe otherwise, the length of the genetic map for chromosome V is due for a considerable extension as new genes are located in the short arm to the left of A_2. Three-point backcross data for $Bm_1 - Pr - V_2$ are available. Bm_1 is so close to the insertion region that we can consider the amount of crossing over between $Bm_1 - Pr$ essentially equal to that between the spindle fiber and Pr. Let us calculate, as best we can, the approximate length of the genetic map for the long arm of chromosome V. The three-point backcross data give the following recombination values: $Bm_1 - Pr = 22.3$ percent, $Pr - V_2 = 43.4$ percent. According to HALDANE'S (1919) table for estimating the map distance from recombination percentages the map distance for the $Bm_1 - Pr$ interval is 24.5 units and that for the $Pr - V_2$ interval is 60.8 units. The sum 85.3 units gives an estimate of the map distance from the insertion region to the V_2 locus but we do not know how far removed V_2 is from the end of the long arm. Since the two arms of chromosome V are nearly equal it appears that the length of the genetic map for this chromosome will exceed 170 map units.

TABLE 1

Ratio of A_2:a_2 *seed from backcross of* $\begin{array}{c} a_2 \\ \hline A_2 \\ \hline \underline{A_2} \end{array} \times \begin{array}{c} a_2 \\ \hline \underline{a_2} \end{array}$.

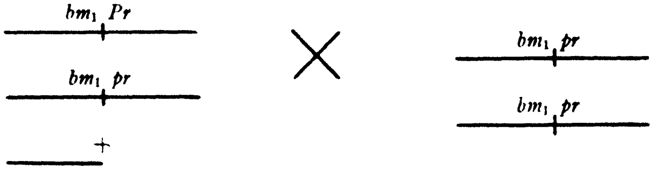
PEDIGREE OF FRAGMENT PLANT		A_2 SEEDS	a_2 SEEDS	PERCENT RECESSIVES
2524	6	187	87	31.8
2524	14	166	66	28.4
2525	9	206	118	36.4
2525	6	228	130	36 3
2525	3	59	25	29.8
2524	25	174	77	30.7
2525	2	117	66	36.1
2525	8	92	44	32.4
2524	29	97	47	32.6
3117	a	195	111	36.3
Total		**1521**	**771**	Aver %=**33.6**

In the course of the studies with the fragment several plants were obtained which carried either recessive a_2 or bm_1 in the two normal chromosomes V and the dominant allele in the fragment chromosome. Considering the bm_1 locus these plants were of the following constitution: $bm_1Pr/bm_1pr/Bm_1$ and for the a_2 locus had $a_2pr/a_2pr/A_2$ constitution. When a plant carrying the bm_1 gene was used in reciprocal crosses with a recessive individual the following results were obtained:

From the above data where the fragment was used as the pollen parent it is evident that even though the fragment plant was Bm_1 in phenotypic appearance it bred true for bm_1 excepting those cases where fragment pollen functioned or where a crossover had occurred which transferred the Bm_1 allele to a normal chromosome.

The following calculations can be made from the data in table 2. (1) The percentage of functioning eggs carrying the fragment chromosome was 33.

TABLE 2



FRAGMENT PLANT AS FEMALE	
2N PROGENY	2N+FRAGMENT PROGENY
$Pr\ bm_1$—62	$Pr\ Bm_1$—31
$Pr\ Bm_1$—1	$pr\ Bm_1$—35
$pr\ bm_1$—64	$wh\ Bm_1$—19
$wh.\ bm_1$—45	
FRAGMENT PLANT AS MALE	
2N PROGENY	2N+FRAGMENT PROGENY
$Pr\ bm_1$—146	$Pr\ Bm_1$—5
$Pr\ Bm_1$—1	$pr\ Bm_1$—1
$pr\ bm_1$—109	$wh\ Bm_1$—2
$pr\ Bm_1$—1	
$wh.\ bm_1$—123	

The above crosses were segregating for colored and colorless aleurone. The colorless (wh.) class was included because of the bm_1 data.

(2) The frequency of crossing over between the fragment chromosome and one of the normal chromosomes in the Bm_1-insertion region was .5 percent. (3) Two percent of the functioning pollen from the fragment plant was hyperploid for the fragment chromosome. Incidentally the transference of the Bm_1 gene from the fragment chromosome to one of the normal chromo-

somes could be used as cytological proof of genetic crossing over if such evidence were needed after the conclusive work of STERN (1931) and CREIGHTON and McCLINTOCK (1931). Obviously a more critical proof would be the transference of the recessive bm_2 allele from a normal chromosome to the fragment chromosome. Such crossovers have occurred in other progenies.

More extensive data from reciprocal crosses similar to the above were obtained for the A_2 locus. The percentage of fragment eggs was 33. The percentage of functioning fragment pollen was 1.2 and the amount of crossing over, based on a thousand individuals, between the fragment chromosome and the normal chromosomes in the A_2-insertion region interval was six percent. JENKIN'S (1934) data places A_2 and Bt_1 7 units apart. Therefore the map distance from A_2 to the insertion region is not more than 7 units. The frequency of crossing over between the fragment and the normal chromosome in the A_2-insertion region interval was six percent. It would seem then that the frequency of crossing over in this interval was the same in disomes as in fragment plants But the fragment is associated with the normal chromosome in approximately 50 percent of the microsporocytes. Therefore the cross over value of six units should be doubled. This indicates that crossing over for this interval, when the fragment is associated with the two normal chromosomes, occurs considerably more frequently in fragment plants than in diploids.

The results obtained from this study of the fragment chromosome agree with previously known facts concerning the order of genes in this chromosome. McCLINTOCK (1932) placed the Bm_1 locus in the short arm near the insertion region from her work with ring-shaped fragments. Moreover, cytogenetic studies with a reciprocal translocation (RHOADES 1933c) indicated that Bt_1 and Bm_1 were both near the insertion region of chromosome V. STADLER (1935) obtained a deficiency for the V_3 locus. This deficiency occurred in the long arm of the chromosome. In addition to substantiating the above, the fragment studied places the two closely linked genes Bt_1-Bm_1 (1–2 units apart) on opposite sides of the insertion region. Moreover it definitely places six loci in the long arm and two in the short arm of chromosome V.

When plants of $bm_1Pr/Bm_1pr/Bm_1$ constitution were used as the pollen parent in a backcross the following data were obtained: 515 $Pr\ bm_1$:568 $pr\ Bm_1$:383 $Pr\ bm_1$:386 $pr\ bm_1$. These data give a recombination value of 41.5 percent for the $Pr-Bm_1$ interval. Diploid sister plants had a crossover value of 22.3 percent for the same region. This large and highly significant increase in the amount of crossing over in the long arm of chromosome V in fragment plants is inexplicable, as somewhat comparable data from Drosophila showed no increase in crossing over in that arm which was not represented in the duplicating fragment (RHOADES 1931). Unfortunately only the *Pr*-insertion region interval was involved in the present studies. More data on this problem will be obtained later.

SUMMARY

1. A plant which was intermediate in appearance between the trisomic and disomic individuals occurred in the progeny of a plant trisomic for chromosome V. This exceptional plant carried in addition to the normal complement of 20 chromosomes a fragment of chromosome V. The fragment chromosome consisted of the entire short arm of chromosome V with a terminal spindle fiber insertion region.

2. The cytological behavior of this fragment chromosome was studied at pachytene and metaphase in microsporocytes. The fragment chromosome was associated with the two normal chromosomes in approximately one-half the cells. Segregation of the three members of the trivalent groups was not random, but in such a manner that the fragment chromosome usually accompanied one of the normals when it passed to a pole.

3. The spindle fiber insertion region of the fragment chromosome was observed to be split or double in those cells where it was lying free in the nucleus as a univalent. The equational split into two chromatids was clearly evident.

4. The V_2 Vs Pr V_{12} V_3 and Bt_1 loci were located in the long arm of chromosome V. The Bm_1 and A_2 loci were located in the short arm of the chromosome.

5. Pollen hyperploid for the fragment chromosome occasionally functioned in competition with haploid pollen.

6. Crossing over in certain regions was apparently more frequent in fragment plants than in diploids.

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FACTORS DETERMINING CONJUGATION IN PARAMECIUM AURELIA

I. THE CYCLICAL FACTOR: THE RECENCY OF NUCLEAR REORGANIZATION

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INTRODUCTION

IN *Paramecium aurelia* a number of factors interact to determine the occurrence of conjugation. Most attention has in the past been given to the role of environmental conditions. In this paper an internal factor is dealt with: nuclear reorganization of the type occurring both at endomixis and at conjugation As will be shown, the relation of these nuclear processes to the subsequent occurrence of conjugation is, in the one stock of *P. aurelia* investigated, very different from what is commonly assumed.

MAUPAS (1889) held that conjugation could occur—or, at least, could result in viable exconjugants—only during the period of sexual maturity. This period was assumed to begin long after the last conjugation and to end when the degenerative alterations characteristic of "senescence" began. MAUPAS' conception of sexual maturity was extended to *P. aurelia*, without direct evidence, as a result of his investigations of other species. Indeed, direct study of *P. aurelia* yielded an embarrassing fact which led MAUPAS to remark that under conditions not yet understood, the normal period of "youth" might be abbreviated in certain species. The fact was the existence of two macronuclei in each of the two members of one pair of conjugants (fig. 33, Pl. 13, loc. cit.). He suggested the possibility that these conjugants might still be in the process of reorganization from a previous conjugation. Subsequently, similar but more convincing observations of this kind have been reported in *P. caudatum* (DOFLEIN 1907; KLITZKE 1914), in *P. multimicronucleatum* (MÜLLER 1932), and in other genera. Nevertheless, in one form or another, many workers down to the present time have held that between two successive conjugations a long period of vegetative multiplication must intervene. Thus, HERTWIG (1889 and later papers) maintained that by long exercise of vital functions the volume ratio of the nucleus to cytoplasm was disturbed and required conjugation or an equivalent process to rectify it. According to this view, the disturbed nucleoplasmic ratio led to a high and increasing rate of fission; and the overdriven life activities led to conjugation. SIMPSON (1901) supported MAUPAS in his report of failure to induce conjugation in *P. aurelia* during the "period of puberty." However, details are lacking and presumably there were no contemporary "mature" controls in which conjugation was induced.

On the other hand, the falsity of this conception of the place of conjugation in the "life cycle" of *P. aurelia* has been clearly demonstrated. JENNINGS (1910) showed for one race that the interval between successive conjugations could regularly be reduced to two or three weeks, sometimes less. In one instance, the interval was reduced to five days and four fissions. Other races, however, could not be induced to yield such short intervals, some conjugating only twice in about two years. JENNINGS held that the minimum interval between conjugations depends upon how much time is required to bring about the three requisite successive stages of starvation, abundant feeding, and decline in multiplication; and that this can be done quickly in some races, but requires a long time in others. Another foundation of the Maupasian sexual maturity conception was completely blasted by WOODRUFF (1908 and later papers). He showed beyond reasonable doubt that under adequate cultural conditions a "life cycle" was nonexistent. At last reports (1932), his stock had been cultivated for 25 years with no indication of a cycle; for eight of these years (5071 fissions) the occurrence of conjugation in the main culture was definitely precluded by means of the daily isolation culture technique. Not only did he show that conjugation was unnecessary, but repeated attempts to induce conjugation were successful only twice: once after more than 6½ years of culture (at about the 4,100th generation) and once after 13 years of culture (after more than 8,000 generations). Further evidence on the periodicity of conjugation was supplied by HOPKINS (1921), who found intervals between successive conjugations as low as 11, 15, 18, 21, 26, 27, and 34 days in some stocks, and as high as three months in other stocks. He reported that the periodicity was very different in different stocks, varying from about three weeks in some to about six weeks in others, and that there was no evidence of periodicity in other stocks. HOPKINS further maintained that the tendency to conjugate was gradually lost under prolonged conditions of rapid growth and could be restored only by a longer or shorter period of dormancy. It appears difficult to reconcile this with WOODRUFF'S experience.

In addition to these reports on periodicity in the occurrence of conjugation in *P. aurelia*, there are in the literature a number of scattered observations whose bearing on the question will become clear only after the experiments that follow have been described. From what has already been reviewed, however, it is clear that in *P. aurelia* there is no "life cycle" including a period of "sexual maturity," that the interval between successive conjugations may be as short as five days, and that periodicity in the recurrence of conjugation differs greatly from race to race.

EXPERIMENTAL PROCEDURE

In the present experiments the stock of *P. aurelia* employed (here designated stock R) was derived from a single individual in the spring of 1929. Numerous investigations upon this stock have already been published by investigators in this laboratory. It consists of many biotypes with diverse characteristics. In typical biotypes, conjugation is often readily induced by the following simple procedure: collect the surplus animals from isolation cultures into fresh culture medium in a small dish and keep them at 31°C. This method was standardized as follows.

The culture medium employed, both for the isolation and the mass cultures, consisted of a lettuce infusion inoculated with the bacterium *Flavobacterium brunneum* and the alga *Stichococcus bacillaris*. In preparing this, powdered desiccated lettuce and redistilled water, in the proportion of 1.5 grams of lettuce to a liter, were boiled for five minutes and filtered at once. The filtrate was distributed into pyrex flasks containing an excess of $CaCO_3$ (Kahlbaum). The $CaCO_3$ brings the pH up to 7.2 and narrowly limits its diurnal variation. The flasks were stoppered with cotton and autoclaved. From this stock fluid, the culture medium was prepared daily by adding to 20 cc of the filtered fluid one 1 mm loop of the bacterium from a 3 to 5 day old slant and three 2 mm. loops of the alga from an 18 day old slant.

The daily isolation cultures were carried on pyrex double depression slides containing two drops (approximately 125 mm^3) of culture medium in each depression. Into the medium in each depression a single paramecium was introduced and kept with its progeny for one day at 27.5° ± 1.0°C. At the end of 24 hours, a record was made of the number of paramecia in each depression, and one paramecium from each depression was transferred to fresh medium on a fresh slide. Of the remaining animals, one or more were stained with acetocarmine to ascertain the nuclear condition, and the rest were either discarded or put into a mass culture with animals from other isolation cultures.

The mass cultures, consisting of animals from the isolation cultures (usually twelve in number), were set up in shallow dishes in 10 drops of culture medium and kept at 31.0° ± 1.0°C. Such cultures were favorable to the occurrence of conjugation and were used for that purpose in all the experiments here reported.

In both the isolation and mass cultures it was necessary, for the purposes of this study, to detect with certainty the occurrence of conjugation; and also of endomixis, since the latter is a factor in the determination of conjugation. This was done as follows:—

Conjugation.—In daily isolation cultures, conjugation rarely occurs. When it does occur, it is only in lines that multiply very rapidly, and then only near the end of the 24 hour interval between observations. Hence it cannot be missed in the course of daily observations. In the experiments here reported, it never occurred in isolation lines. In mass cultures, on the other hand, observations at 24 hour intervals cannot be depended upon; but observations at 12 hour intervals are reliable. Conjugants remain united about eight hours, and different pairs in a conjugating culture begin conjugating at various times over a period of 12 hours or more. Hence, the rule employed was to observe all mass cultures at intervals not exceeding 12 hours.

Endomixis.—In both isolation and mass cultures, the condition of the macronucleus was the criterion of endomixis. Complete disintegration of the macronucleus (into either ribbons or spherical fragments), or fragments plus the lightly staining anlagen of the new macronuclei, were required at some point in the isolation line and among some individuals of a mass culture before concluding that endomixis had occurred. The finding of fragments associated with a whole macronucleus was not, in itself, considered sufficient evidence of endomixis. Actually, very few cases arose in which doubt was possible, and these few were excluded from the experiments.

In isolation cultures, samples of each line were stained every day; the number of individuals stained in each line was at least equal to the number of fissions in that line during the preceding day. Since on the same day different individuals in the same isolation culture frequently differ in their nuclear condition (for example some may be in endomixis, others not), the nuclear condition found in the stained animals is not necessarily the same as in the animal retained to continue the line; but it does definitely indicate the nuclear condition on the preceding day of the common ancestor of the stained animals and of the animals kept alive to continue the line. Thus, if this common ancestor was not in endomixis, its descendents stained the following day would show either no endomixis or very early stages; if the former was in very early endomixis, then the latter would show middle stages of endomixis; if the former was in the midst of endomixis, then the latter would show late stages; and if the former was in late stages of endomixis, the latter would show no endomixis at all. By correlating the observations made on the same line of descent on successive days, it can be definitely ascertained, in practically all cases, whether endomixis has occurred. The rare cases in which doubt is possible were excluded from the experiment.

In mass cultures, the detection of endomixis requires prior knowledge as to whether conjugation has occurred, for both conjugation and endomixis involve similar macronuclear pictures. If conjugation has not occurred, endomixis may be detected by staining representative samples from a culture every day until fully fragmented animals are found. Actually, many cultures were not stained every day. It usually could be foretold whether endomixis would appear in any culture, and if so, on what day it would appear. In such cases, staining of samples was often omitted on the preceding days. If conjugation has occurred in a culture, the detection of endomixis involves deciding whether all the fragmented animals found are exconjugants or whether some are endomictic animals. When the proportion of fragmented animals is nearly the same as the proportion of recent conjugants, it is practically impossible to make this decision. On the other hand, when the proportion of fragmented animals is much greater than the proportion of recent conjugants, then it is clear that endomixis is also in progress.

RELATION OF THE OCCURRENCE OF CONJUGATION TO THE RECENCY OF NUCLEAR REORGANIZATION

In usual biotypes of stock R of *P. aurelia*, conjugation occurs only if endomixis or conjugation has recently taken place. This unexpected relation is demonstrated as follows. If two mass cultures are set up at the same time, in the way above described, one containing animals from isolation lines that have recently undergone endomixis or conjugation, the other containing animals from isolation lines that have not recently undergone endomixis or conjugation, it is found that the former promptly conjugate, while the latter do not.

Such experiments were repeated many times through a period of more than a year, and the results were always the same. There were examined 131 cultures containing descendents of animals that had recently undergone endomixis or conjugation, and all of these quickly yielded conjugants. Of cultures containing descendents of animals that had not recently undergone endomixis or conjugation, 232 were examined; none of these yielded conjugants.

Thus, in the period following endomixis or conjugation, the paramecia undergo a change that is reflected in their reaction to the environmental conditions favoring conjugation. During the first part of this period, they react to the standard conditions by conjugating; but in the later part of the period, under the same standard conditions, they fail to react by conjugating.

At what point does the change in reaction occur? Is the change sudden or gradual? To answer these questions, mass cultures were set up on successive days of the period following endomixis or conjugation. This was managed by carrying a group of isolation lines, all of which had been in endomixis or conjugation the same day, and setting up daily a mass culture of the surplus animals from these. (When any of the isolation lines under-went endomixis again, these were of course dropped from the experiment.) Thus, series of mass cultures were obtained, differing by intervals of one day in the time since they had undergone endomixis or conjugation. Their reaction to the standard conditions favoring conjugation was determined.

In all, there were studied in this way seven series[1] covering the period following endomixis, and one series following a previous conjugation. In three of the series (these were for the period succeeding endomixis), the change in reaction occurred suddenly. Up to a certain time conjugation occurred abundantly in the mass cultures; beyond this time it did not occur at all. In the other five series, there was an intermediate period in which conjugation occurred in only a small proportion of the animals of a culture.

The times at which the change in reaction occurred differed in the different series. The period of abundant conjugation lasted from five days up to twelve days in some series. That is, in some of the series, abundant conjugation occurred only in mass cultures set up on any of the first five days after the climax of endomixis, while in other series it occurred in cultures set up during a longer period after endomixis—up to twelve days in one series.

The period of scanty conjugation also varied. In two series it lasted up to the twelfth day; in one series up to the fourteenth day; in one series up to the sixteenth day. In the series begun with exconjugants it lasted up to the twentieth day. In this period of scanty conjugation only a dozen pairs, or fewer, would be found in any of the densely populated mass cultures. While these few animals were conjugating, many of the nonconjugating individuals were undergoing endomixis.

In the mass cultures set up at longer periods after endomixis or conjugation, conjugation fails to occur, but endomixis occurs in an even higher proportion of the individuals. This proportion increases as the time since the previous endomixis or conjugation increases, until finally it reaches 100 percent. These relations will be set forth in detail in a later paper.

The further history of these late cultures, in which a new endomixis occurred, is of great interest for the relation of the occurrence of conjugation to the occurrence of a previous endomixis. Of such cultures 124 were studied. Some fresh fluid was added to most of them at the time of endomixis. Such fresh fluid did not cause conjugation at the start of these mass cultures, at a period long after endomixis. Of the 124 cultures, conjugation began in every one within three days after the majority of the animals had undergone endomixis.

In many of these cultures it is entirely clear that the conjugating pairs were the descendents of individuals that had just undergone endomixis. (1) In some cultures, examination shows that endomixis is universal; all the individuals go through it and three days later many of them conjugate. (2) Further, many of the conjugants, if stained in early stages of conjugation, show besides the entire macronucleus (or one just beginning to unravel into ribbons), a number of the pale macronuclear fragments left over from the recently preceding endomixis.

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[1] I am grateful to Dr. Ruth Stocking Lynch for assistance in the study of one of these series.

The experiments and observations described above demonstrate that, in this stock of *P. aurelia*, conjugation may readily be induced immediately after the completion of endomixis, though at other times it does not occur or occurs but scantily. This relation is of practical importance in cases in which it is desired to bring about conjugation. It affords a thoroughly dependable method of obtaining abundant conjugation within 24 hours when a large number of recent ex-endomictics are available. When the number of such individuals is as great as 500 to 1,000, conjugation sometimes begins before all have been collected into the mass culture, and hundreds of pairs will be found within 12 hours.

The fact that the similar processes of endomixis and conjugation thus normally occur so close together appears remarkable. A similar close association may exist between two successive conjugations. To test this, 22 mass cultures were set up with conjugants, there being at the beginning in the different cultures from 7 to 53 pairs. All soon yielded a new set of conjugants. The average interval from the original conjugation to the next one was 3.2 days.

To determine whether conjugations can continue to succeed one another at such short intervals, two series of cultures were followed. In each series, when conjugation occurred in any culture, some of the conjugants were removed to a new dish in fresh fluid to start a new culture. In these series, as in all experiments involving conjugants, only those pairs were used that had been observed to remain firmly united for at least three hours. This avoids the danger of using pairs that do not actually complete conjugation. The two series gave similar results. In one, eleven successive conjugations were induced at intervals of 4, 5, 3, 3, 4, 6, 2, 2, 1, and 1 days respectively. In the other series eight successive conjugations were induced at intervals of 2, 3, 2, 3, 2, 3, and 2 days respectively. There appears no reason to doubt that such series of successive conjugations could be continued indefinitely.

DISCUSSION

The results just described show clearly that in stock R of *P. aurelia* conjugation is readily induced during the several days following conjugation or endomixis, but that if a longer time elapses another endomixis must occur before conjugation can be repeated. In other stocks of the same species these relations do not hold, as will be shown in a later paper. Nevertheless, numerous observations scattered through the literature show that similar relations do hold in at least some other stocks.

It has already been pointed out that the occurrence of very short intervals between successive conjugations was suspected by MAUPAS (1889) and demonstrated by JENNINGS (1910). Similar results have been reported in *P. putrinum* by BÜTSCHLI (1876) and JOUKOWSKY (1898); in *P. caudatum* by DOFLEIN (1907), KLITZKE (1914), and others; in *P. multimicronucleatum* by MÜLLER (1932) and GIESE (1935); and in other genera.

Clear instances of conjugation within several days after endomixis are rarely found in the literature, partly because endomixis was unknown or insufficiently appreciated by workers prior to 1914, and partly because it has been neglected by later workers on conjugation. There is, however, one clear case in the work of WOODRUFF (1914). He reports (p. 237) that the first conjugation in his long cultivated stock occurred on the fifth and sixth days after setting up a mass culture with animals in generation 4,102. Reference to the paper of WOODRUFF and ERDMANN (1914, p. 437) shows that endomixis occurred at generation 4,103. Thus the first conjugation in 6½ years of culture occurred just after an endomixis. In another place (p. 492), WOODRUFF and ERDMANN report that they found endomixis occurring in some animals of a mass culture while others were conjugating. HERTWIG (1889) observed the same thing; and MÜLLER (1932) found similar phenomena in *P. multimicronucleatum.*

Further indirect evidence of the time relations between the occurrence of conjugation and endomixis is to be found in accounts of the periodicity of conjugation. The two or three week periodicity of conjugation reported by JENNINGS for his stock k is the same as the periodicity of endomixis in the present stock R. If the same periodicity of endomixis characterized JENNINGS' stock, its periodicity of conjugation is readily explained. Similarly, the diverse periodicities of conjugation in the different stocks used by HOPKINS (1921) agree with the differences in interendomictic intervals that characterize different stocks, as will be set forth in a later paper. Likewise, the longer intervals found by HOPKINS between conjugations in *P. caudatum* agrees with the greater interendomictic intervals in that species.

There are still other indications of the time relation between endomixis and conjugation: (1) It is often said that the tendency to conjugate is heightened during periods of depression, which are now well known to be periods of endomixis. (2) The common method of inducing conjugation (JENNINGS 1910) is one which would be expected to induce endomixis just before conjugation. The method consists of bringing about two periods of decline in food supply with an intervening period of abundant feeding. Conjugation is expected to occur during the second decline; the function of the first decline is probably to induce endomixis. On this view, if endomixis occurred recently, the *first* decline in food supply should yield conjugants; and this has in fact been reported by investigators using this method (SONNEBORN and LYNCH 1934, p. 10; RAFFEL 1930, p. 300).

There are in the literature not only evidences that conjugation occurs at definite times with relation to prior nuclear reorganization, but also evidence that there are times when conjugation cannot be induced. Long observation of any one stock shows that a standard set of inducing conditions will yield conjugants at certain times but not at other times (JENNINGS 1910, HOPKINS 1921, and others). Such observations have led these investigators to suggest that an unknown internal factor plays a role in the determination of conjugation, and this factor may well be the one described in this paper.

What event, process, or substance controls the tendency to conjugate in this stock of *P. aurelia?* Since either conjugation or endomixis serves to put the organisms in a condition in which conjugation can be induced, it may be concluded that the differences between these two processes are not of importance in this connection. Hence, reduction of chromosome number, the introduction of new chromatin from a different animal, and the formation of a syncaryon may be excluded at once. On the other hand, the internal factor that makes conjugation possible must be common to both endomixis and conjugation. There appear to be three such common things from which to choose: (1) disintegration of the old macronucleus; (2) reintegration of a new macronucleus; (3) resorption of the fragments of the old macronucleus and the supernumerary micronuclei. Of these three, two can be eliminated in the light of additional known facts.

The lost tendency to conjugate is restored before animals have reached the stage of endomixis in which resorption and reintegration of the nuclei occur. Many pairs have been observed in which one or both members are in the midst of endomixis. The endomictic "conjugants" are frequently in the stage of endomixis in which anlagen of the new macronucleus have just begun to enlarge and still take practically no chromatic stain; they may even be at the climax of endomixis, fully fragmented but lacking anlagen of the new macronucleus. In such cases, the normal mates show either a whole macronucleus with no fragments, or a macronucleus at the very earliest stage of ribbon formation. These unusual pairs have been found also by others. Some of the earlier mentioned cases of supposed reconjugation of recent conjugants may really be of this type; MÜLLER (1932) definitely states that some of his pairs of *P. multimicronucleatum* contain one member in the midst of endomixis. In my own work, such pairs have occurred under a definite set of conditions. As described above there was in some experiments a period of scanty conjugation, beginning in some series as early as the eleventh day and lasting in some up to the twentieth day after the last endomixis or conjugation. During this period of scanty conjugation, many animals in a mass culture would go through endomixis at the time when a few others conjugated. In these cultures some of the pairs included animals in the midst of endomixis. What nuclear processes occur in such animals is at present unknown but is under investigation in this laboratory. But the fact that animals in early stages of endomixis will unite as if to conjugate shows that the important thing done by endomixis in preparing animals for conjugation is accomplished early in the process, by the time it reaches the climax. Restoration of the tendency to conjugate must therefore be associated with the prior breakdown of the old macronucleus.

On this view, conjugation is possible so long as an old integrated macronucleus is absent; that is, from the moment it disintegrated, at the climax of endomixis and early in conjugation, until the new macronucleus then formed has lived to be a week or so old. There are two ways of interpreting this relation between the macronucleus and the tendency to conjugate. Either, as the macronucleus ages, it inhibits conjugation; or, when the old macronucleus disintegrates, it discharges into the cytoplasm an enzyme or catalyst which makes conjugation possible. Between these alternatives, a decision can be made on the basis of the relation between rate of reproduction and duration of the tendency to conjugate. There should be a precise inverse proportionality, if the second alternative is correct. Loss of activity by an enzyme or catalyst is presumably due to its dilution below a threshold concentration, and such a point would be reached after a definite number of fissions, irrespective of the time involved. Furthermore, if the tendency to conjugate is dependent upon the disintegration of the old macronucleus, this can be demonstrated by finding the earliest moment after conjugation at which reconjugation is possible in *P. aurelia* and *P. caudatum*. Disintegration of the macronucleus occurs *during* conjugation in the former, and some time *after* it in the latter. Hence, exconjugants should be able to reconjugate at once in *P. aurelia*, but not in *P. caudatum*.

SUMMARY

In stock R of *P. aurelia*, conjugation may be induced by collecting the surplus animals from isolation cultures into fresh culture medium in a small dish and keeping them at 31°C. But not all such cultures conjugate. The determining factor here is the recency of nuclear reorganization. When the culture consists of descendents of animals that have recently undergone endomixis or conjugation, it quickly yields conjugants; but when the culture consists of descendents of animals that have *not* recently

undergone endomixis or conjugation, it will not yield conjugants until after another endomixis has occurred. Response to the conditions favorable for conjugation is of three types, each type characterizing a period of the time following nuclear reorganization. (1) During the first period, varying in extent from the first five to the first twelve days, cultures respond with abundant conjugation; (2) during the second period, extending in some series from the eleventh up to the twentieth day after nuclear reorganization, cultures yield very small proportions of conjugants, or none at all; (3) subsequent to this, in the third period, all cultures completely fail to conjugate but endomixis occurs abundantly instead. Cultures set up in the third period will conjugate readily *after* endomixis has occurred in them.

Not only can two successive conjugations occur with a short interval between them, but long series of successive conjugations are inducible with repeated intervals of only one or a few days. Under certain conditions, individuals in endomixis (climax stage and later) unite as if in conjugation. Hence the process in endomixis that restores the tendency to conjugate must occur early. It must also be the same as a process occurring during conjugation. Disintegration of the old macronucleus is apparently the only process that satisfies these two conditions. A further means of testing the validity of this conclusion is proposed.

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FACTORS DETERMINING CONJUGATION IN PARAMECIUM AURELIA

II. GENETIC DIVERSITIES BETWEEN STOCKS OR RACES

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INTRODUCTION

IN TYPICAL biotypes of the Johns Hopkins stock R of *P. aurelia*, SONNEBORN (1936) has shown that, when surplus animals from isolation lines are collected in a small mass culture and placed at 31°C, conjugation invariably occurs if the animals have recently been through a nuclear reorganization (that is, endomixis or conjugation), but never at other times. In the present paper we contrast the behavior of this stock with that of the Yale stock, cultivated since 1907 by WOODRUFF, which responds very differently to these same conditions.

In *P. aurelia*, racial diversities in the tendency to conjugate have been reported by JENNINGS, WOODRUFF, HOPKINS, and BALL, but their work has been criticized by ENRIQUES, ZWEIBAUM, and the CHATTONS. JENNINGS (1910) found a race k, which was observed to go through 20 epidemics of conjugation in about two years; but during this period race C2 conjugated only a few times, and races c and i only twice. Long continued, systematic and varied attempts to induce conjugation in races c and i invariably failed, but similar attempts in race k were always successful if two or three weeks had elapsed since the last conjugation. WOODRUFF (1912) reported similar results; in some stocks conjugation occurred readily, but in his stock cultured since 1907 conjugation could be obtained only twice, once in 1913 (WOODRUFF 1914) and once in 1920 (WOODRUFF 1921). The most extensive study along these lines was made by HOPKINS (1921). He found marked differences among 13 races, in three respects. (1) Differences in the tendency to conjugate: under similar conditions, some races conjugated frequently, other races only rarely, some not at all. (2) Differences in the conditions required for conjugation: some races conjugated in large stock cultures without special experimental treatment; other races would not conjugate under these conditions, but did so when transferred to small watch glasses with a rapidly diminishing food supply; still other races would not conjugate even under these conditions, but could be made to conjugate when this treatment was preceded by a longer or shorter period of "dormancy." (3) Differences in the periodicity of conjugation: some races showed no periodicity, others conjugated fairly regularly at intervals of about six weeks, still others at intervals of about three weeks. BALL (1925) added further slight evidence of the same sort, though his main work was on *P. caudatum.*

On the other hand, ZWEIBAUM (1912) and the CHATTONS (1931) have denied the existence of racial differences in the tendency to conjugate. These investigators were able to induce conjugation at will in *P. caudatum* under certain conditions, but not under other conditions; from this they concluded that the diverse results obtained by other investigators on different stocks were actually due to uncontrolled diversities in the environmental conditions. There are, however, several reasons why their conclusion cannot be accepted. In the first place, the production of diversity in the tendency to conjugate by means of diverse environments in one case is no reason to conclude that the same diversity cannot be produced by diversity of constitution in another case. Secondly, even if it could be shown (which, as yet, has not been done) that all or many stocks of a species could be induced to conjugate under one set of conditions, genetic differences might still be demonstrated if, under another set of conditions, some stocks conjugate and others do not. Thirdly, so far as can be learned from their published accounts, ZWEIBAUM (1912) and the CHATTONS (1931) each worked on but a single stock of *P. caudatum*, so they have had no opportunity to encounter racial diversities. Fourthly, the results of the CHATTONS are at variance in a fundamental respect with the results of ZWEIBAUM. ZWEIBAUM found that an essential prerequisite to the induction of conjugation in his stock was a period of starvation ("disette") of 5 or 6 weeks, during which a nutritive equilibrium exists. The CHATTONS, however, found that this was unnecessary in their stock. Thus, the two main proponents of the view that genetic factors play no role in the induction of conjugation have themselves shown that their two stocks differ in the conditions required for conjugation. Finally, HOPKINS (1921) and BALL (1925), using ZWEIBAUM'S methods on *P. caudatum* (as well as on *P. aurelia*), were unable to support his conclusion. They found that while his methods were successful with some stocks, they failed with others, and that they were unnecessary in still other stocks, as the CHATTONS also found. Their work, therefore, agrees with the earlier work of JENNINGS (1910), WOODRUFF (1912), and CALKINS and GREGORY (1913) on the same species, as well as the papers cited in the preceding paragraph on *P. aurelia.* In addition to these investigations on *P. aurelia* and *P. caudatum*, WENRICH and WANG (1928) presented evidence for racial differences in tendency to conjugate in *P. calkinsi.*

Thus ZWEIBAUM'S contention has been experimentally disproved. That of the CHATTONS is in little better case, but an investigation directed precisely upon the point which they make, and that shall adequately test it, will be worth while. Such an investigation is here presented. The CHATTONS maintain that differences in bacterial flora are the basis of apparent racial differences in tendency to conjugate. It is required, therefore, to compare different stocks under identical bacterial conditions.

EXPERIMENTAL RESULTS

For this purpose, we selected two stocks reported to be very diverse in their tendencies to conjugate. The first was the Johns Hopkins stock R in which conjugation could invariably be produced under the conditions described by SONNEBORN (1936). The second was the Yale stock in which conjugation has occurred only twice in 29 years (WOODRUFF 1921). (We are grateful to Professor Woodruff for his kindness in sending us a strain of this stock.) The carefully controlled method of cultivation, using but one species of bacterium and one species of alga, and the methods of inducing and detecting conjugation and endomixis were all fully described by SONNEBORN (1936) and need not be repeated here.

The two stocks were compared, under identical environmental conditions, in the following way. A large group of daily isolation lines of each stock was cultivated on pyrex depression slides from February 15 until March 30, 1935. Each day the surplus animals from the isolation cultures were collected into small mass cultures, which were kept at 31°C and observed for conjugants at not more than 12 hour intervals. All mass cultures were observed for at least five days (when multiplication had practically stopped); and those cultures in which 75 percent or more of the animals went into endomixis were fed again and observed for at least four more days until after multiplication had again practically stopped. Altogether, there were under observation 179 mass cultures of the Yale stock and 187 mass cultures of the Johns Hopkins stock R. *Not a single pair of conjugants could be found in any of the cultures of the Yale stock; but conjugation occurred in every one of the cultures of the Johns Hopkins stock R.*

Although both stocks were cultivated under identical conditions and although pure cultures of but one species of bacterium (*Flavobacterium brunneum*) were inoculated into the culture fluid daily, it was still possible that different contaminating species of bacteria had gotten into the cultures of the two stocks. To equalize such contamination, if it had occured, lines of the Johns Hopkins stock R were transferred to fluid in which the lines of the Yale stock had lived for 24 hours, and vice versa. During the days following this exchange of fluids, 57 mass cultures were prepared as before. The results with these cultures were exactly the same as before: *all cultures of the Johns Hopkins stock R conjugated, but none of the cultures of the Yale stock did.* Their difference in the tendency to conjugate cannot, therefore, be due to differences in bacterial flora. The clear-cut, constant, and many times repeated difference found between these two stocks demonstrates definitively that they differ genetically in their tendency to conjugate. Under the same environmental conditions, they invariably respond differently. The criticism of the CHATTONS, like that of ZWEIBAUM, is thus unfounded, and racial differences in tendency to conjugate do in fact exist within a single species of Paramecium.

SUMMARY

Under carefully controlled environmental conditions, the Johns Hopkins stock R of *P. aurelia* can invariably be induced to conjugate; under identical conditions, the Yale stock of the same species cannot. The contention of the CHATTONS (1931) that such differences are not racial, but due to differences in bacterial flora, is shown to be invalid.

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STUDIES ON THE INHERITANCE OF PERSISTENCY[1]

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INTRODUCTION

THE ability to lay late in the fall at the close of the first laying year is very desirable in breeding for egg production. GOODALE and SANBORN (1922) pointed out that high persistency is one of the essential characters in heavy laying Rhode Islands Reds. HAYS and SANBORN (1926) showed an intimate correlation between persistency and egg production. HAYS and SANBORN (1927a) found the net correlation between persistency and [illegible] production to be higher than between any other fecundity char[illegible]d and annual production. HAYS and SANBORN (1933) also re[illegible]ding from females with persistency greater than 365 days gave [illegible] having superior fecundity. JULL (1932) also showed the importance of high persistency in relation to egg production.

Studies on the mode of inheritance of persistency are very limited. HURST (1925) stated that high persistency behaves as a simple recessive to low persistency. HAYS (1927b) presented data on the inheritance of high persistency in Rhode Island Reds. In this early study on production-bred birds, the dividing point between high and low persistency was arbitrarily placed between 314 and 315 days from first pullet egg. Persistency was also limited to a 365 day period following the first pullet egg. The studies reported here include the production-bred birds hatched from 1917 to 1930, the exhibition-bred birds hatched in 1930 and F_1 and F_2 hybrids and backcrosses hatched in 1930, 1931, 1932, and 1933. In these later studies the persistency period may continue beyond the 365-day laying year until the individual shows a 30-day cessation of production which is assumed to indicate the annual molt. Persistency within the first laying year may likewise be terminated by a pause of thirty days or more.

CHARACTER OF THE DIFFERENT POPULATIONS

A total of 910 production-bred pullets hatched from 1917 to 1930 may be considered a representative sample of the production stock used for hybridization. Figure 1 shows the frequency distribution of this population with respect to length of biological year. The data fail to show a normal distribution unless five individuals with persistency between 185 and 214 days are omitted. Omitting these five, the distribution by the χ^2 test gives a probability of .2498 which means that in one case out of four a random sample would give deviations as great as or greater than those observed.

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FIG. 1

BIOLOGICAL YEAR – DAYS

910 PRODUCTION – BRED BIRDS

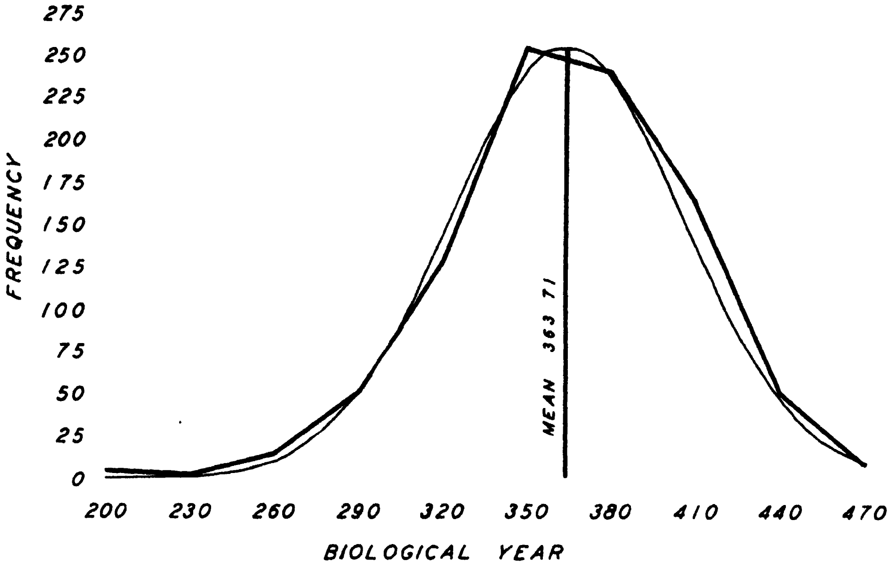
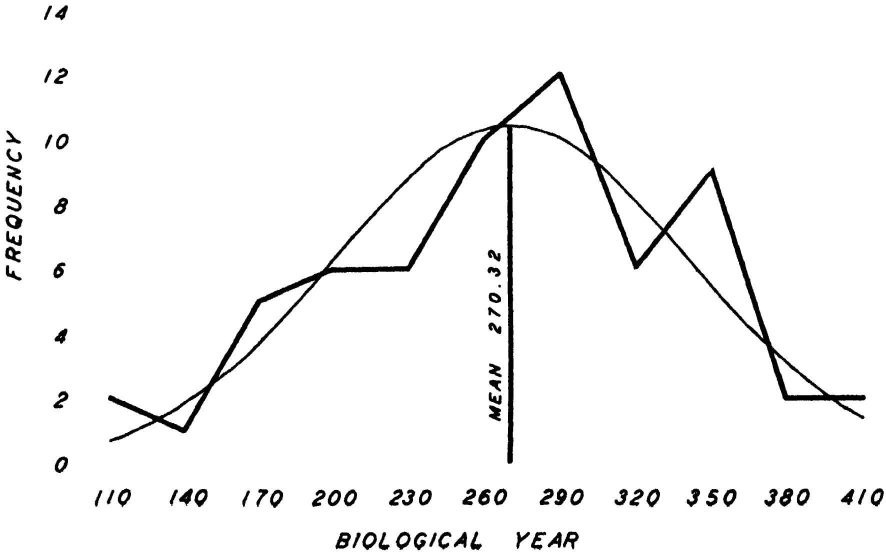


FIG 2

BIOLOGICAL YEAR – DAYS

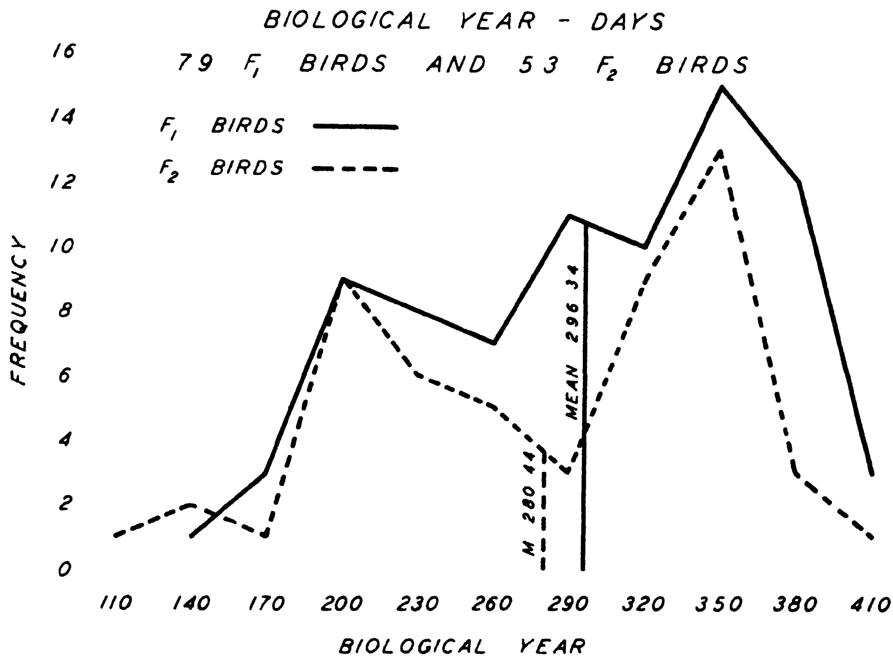
61 EXHIBITION BIRDS – 1930



The generation of exhibition birds hatched in 1930 consisted of 61 individuals with a mean biological year of 270.32 days. Figure 2 gives the frequency distribution of this population for biological year. This distribution is very close to normal and the χ^2 test for normalcy gives a value of P of .6655.

First and second generation crosses are presented in figure 3. The frequency distribution for the F_1 generation shows a wide scatter as might be anticipated when a cross is made between two lines neither of which is homozygous for the character in question. There is an indication, however, of the dominance of high persistency.

FIG 3



BIOLOGICAL YEAR - DAYS

79 F_1 BIRDS AND 53 F_2 BIRDS

The F_2 generation clearly shows a bimodal distribution for length of biological year with modes at about 200 days and 350 days. The graph demonstrates that two populations with rather characteristic length of biological years are concerned. An inspection of this graph suggests that the dividing point between the two populations lies in the neighborhood of 270 days.

Figure 4 gives the frequency distribution of the 23 F_2 birds that had a biological year below 270 days. This population has a mean of 203.63 days and the χ^2 test for normalcy of distribution gives the value of P as .3885 which is satisfactory evidence that the distribution is normal.

FIG. 4

BIOLOGICAL YEAR - DAYS

23 F_2 BIRDS - UNDER 270 DAYS

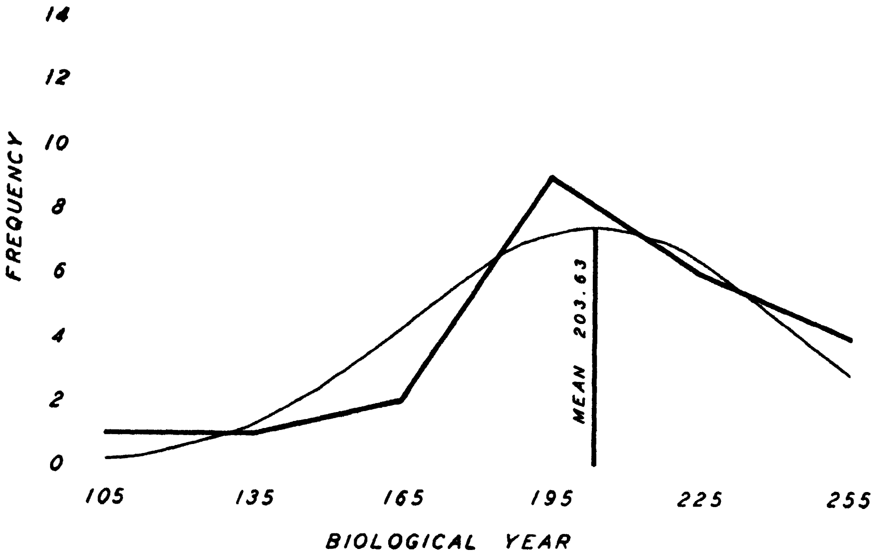


FIG 5

BIOLOGICAL YEAR - DAYS

30 F_2 BIRDS - OVER 270 DAYS

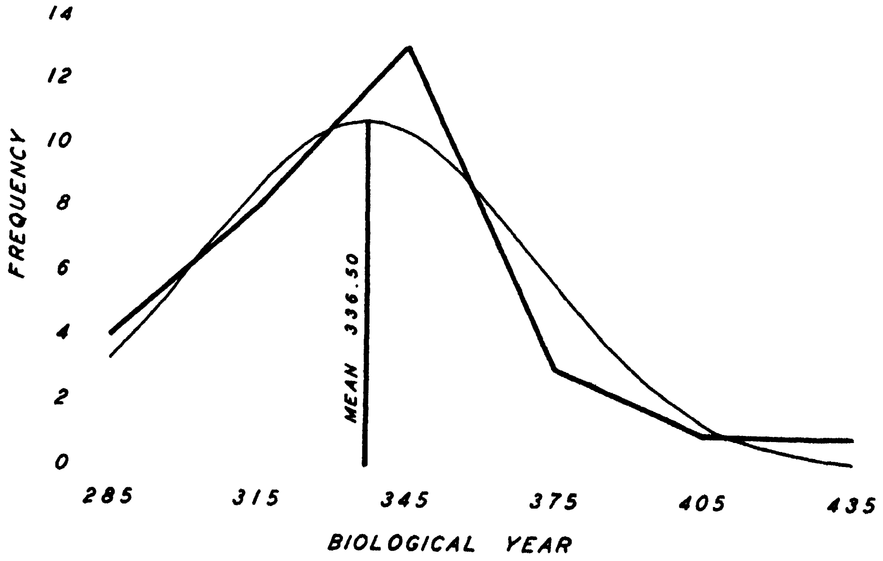


Figure 5 includes the 30 F_2 birds with a biological year of 270 days or more. The mean persistency of this population is 336.50 days. By the χ^2 test the value of P is .2343 showing that this group of persistent layers represents a normal distribution. The fact should be stressed in this connection that the arbitrary separation of the F_2 population into low and high persistency classes at 270 days will operate to include some individuals around this dividing point in the wrong phenotype.

RESULTS FROM CROSSES

For analytical and practical purposes the dividing point between high and low persistency phenotypes appears to occur at close to 270 days. The former figure of 315 days used by HAYS (1927) is not substantiated in crosses where an essentially low persistency line of exhibition birds is crossed with a highly persistent line of production birds.

Some specific examples of the ratios may next be considered. In 1930 an exhibition male No. J711 was mated to four production females all of which were phenotypically highly persistent. From these matings 45 F_1 daughters have complete persistency records. The data show 24 highly persistent to 21 low in persistency with an expectation of equal numbers if one dominant gene is concerned. The male evidently lacked gene *P* and the females were heterozygous. This male was shown to lack the gene for high persistency by mating to his F_1 daughters. Unfortunately only a small number of daughters from three matings had complete persistency records. These showed 2 high to 5 low where the expectation was 3.5 to 3.5. The maternal backcross obtained by mating the original production females to an F_1 son gave 10 high to 2 low where the expectation was 9 to 3. A cross of production males to exhibition females that were phenotypically high gave 23 F_1 daughters in the proportion of 19 high to 4 low where the expectation was 17.25 to 5.75. A production male K56 mated to F_1 females that were high in persistency gave 20 high to 4 low where the expectation was 18 to 6.

A production male mated to persistent F_2 females gave 15 high to 4 low daughters and the expectation was 14.25 to 4.75. An exhibition male mated to persistent F_2 females gave 8 high to 4 low where the expected was 9 to 3.

One exhibition male, K718, when mated to low persistency hens gave proportions of highly persistent daughters indicating that he carried one gene for high persistency. Reciprocal crosses between the two lines gave the same results confirming earlier findings at this Station that high persistency depends in inheritance upon a single dominant autosomal gene *P*.

SUMMARY

Results from crossing high and low persistency lines of Rhode Island Reds show that the dividing point between high and low persistency phenotypes lies close to 270 days. That high persistency depends in inheritance upon a single dominant autosomal gene is confirmed. In breeding operations it is recommended that the standard for selecting breeding stock for high persistency be placed well above a 270 day minimum because of overlapping in phenotypes.

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THE INHERITANCE OF TAILLESSNESS (ANURY) IN THE HOUSE MOUSE

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INTRODUCTION

THE short-tailed (Brachyury) mutant form of the house mouse, discovered by DOBROVOLSKAIA-ZAWADSKAIA (1927a), behaves as a dominant to the normal or wild type. The homozygotes show characteristic abnormalities (CHESLEY 1932, 1935) and die *in utero* about eleven days after fertilization.

From the original short-tailed (Brachy) stock following outcrosses to wild mice, three substrains have arisen, each characterized by complete absence of the bony tail (anury). These have been described by DOBROVOLSKAIA-ZAWADSKAIA and KOBOZIEFF (1927b). These authors (1932) and KOBOZIEFF (1935) have reported that tailless mice of each of these strains produce only tailless mice when bred to members of the same strain. Crosses between members of different strains are said to produce both tailless and normal progeny, although complete data on such crosses have not been published. Because of this peculiar behavior, ZAWADSKAIA and KOBOZIEFF suggested that the tailless strains may contain balanced lethals. This suggestion rested on analogy, since from the evidence available it was not possible to identify the lethals involved nor to specify what conditions held the lethals in association.

Our interest in the developmental action of lethal genes led us to undertake a further analysis of the tailless strains in order to isolate the genes involved and to study their effects on the embryos. The results of this analysis show that mice of one of the tailless strains (Agouti or A line of

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[1] These experiments were being carried on in this laboratory by Dr. Chesley, and most of the observations (except those on line 29) were made by him. Before Dr. Chesley's death in February 1936, we had had frequent consultations on the results, and I was sufficiently familiar with the data and with Dr. Chesley's working hypotheses to be able to continue the experiments as originally planned. Although the major part of the data and of the interpretations had been obtained by Dr. Chesley, he had not intended to publish them until after further evidence had been obtained concerning (1) the morphological characters of the new type of dead embryos found (t^0t^0 p. 530), (2) the difference in the segregation ratios from males and females containing the t^0 gene which he thought required an examination of the spermatogenesis of T/t and $+/t^0$ males, (3) the possibility of crossing over between T and t^0 since he was not convinced that these genes were alleles. Since some time may elapse before the first two problems can be adequately studied, I have thought it best to record the results obtained and to assume responsibility for the form of publication and the conclusions drawn. I wish, too, to record my respect and admiration for Dr. Chesley as student, colleague and friend, and my regret that a scientific career that had begun so auspiciously should have ended so early. L. C. D.

ZAWADSKAIA) are heterozygous for two lethals, one the previously known Brachyury (T), the other a recessive (t^0) which in combination with T produces taillessness. T and t^0 are probably alleles or so located as to show no crossing over. In this report we shall summarize the genetic analysis of this tailless strain. Preliminary evidence is reported showing that t^0 homozygotes die very early in development, shortly after implantation. It is hoped that a more complete description of the morphology and development of the new lethal type can be made later.

MATERIALS

Dr. ZAWADSKAIA kindly brought stock of all three strains to our laboratory in 1933 and placed them at our disposal. We are greatly indebted to her for her cordial cooperation in our work.[2] Because of difficulties encountered in maintaining the stocks, it was necessary to outcross two of them (lines 29 and 19, cf. KOBOZIEFF 1935) to normals and Brachys of a stock previously supplied by Dr. ZAWADSKAIA and to recover the strains by backcrossing the outcross animals to the ZAWADSKAIA tailless strains followed by inbreeding. Although we recovered true-breeding tailless stocks of each of these, it is not certain that the genetic constitution of each is identical with that of the original stocks. The third tailless stock (A line) is descended from Dr. ZAWADSKAIA's stock without outcrossing.

BREEDING EXPERIMENTS

Our first experiments confirmed the reports of ZAWADSKAIA and KOBOZIEFF that each of the tailless lines bred true and was characterized by small litters (table 1). The progeny from lines A and 19 were all completely tailless, no vertebrae having been noted (by palpation) posterior to the

TABLE 1

Results of matings of tailless lines inter se and of crosses between different tailless lines.

PARENTS	REFERENCE	OFFSPRING			LITTER SIZE
		TAILLESS	BRACHY	NORMAL TAIL	
A ×A	This paper	239			3·6
A ×A	KOBOZIEFF (1935)	*63*			*2 7*
29×29	This paper	167			3·8
29×29	KOBOZIEFF (1935)	*194*			*4·1*
19×19	This paper	63			3·9
19×19	KOBOZIEFF (1935)	*34*			*3·4*
A ×19	This paper	9		9	3·6
A ×29	This paper	23		15	5·7
A ×29	KOBOZIEFF (1935)	*14*		*15*	5·8

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[2] For later shipments we are also grateful to Dr. Walter Landauer and Dr. Boris Ephrussi who brought mice to us from Dr. ZAWADSKAIA's laboratory.

sacrum. In line 29, we have found occasional animals with a small bony stump, never containing more than 2 or 3 tail vertebrae. In all lines there is usually a short "tail filament" consisting of skin and connective tissue, probably the remains of the tail which is formed in early embryonic development, but which is destroyed and resorbed, as described for the short-tailed mice (CHESLEY 1934). Many of the animals in each line are sterile, small and mature slowly.

Inheritance of Taillessness in Line A

The first problem was to determine the genetic constitution of the tailless animals in each strain. Reciprocal crosses were made between tailless animals and normals from an inbred stock (Bagg albinos) and between tailless and short-tailed (Brachy) animals of the ZAWADSKAIA parent stock. The progeny of these crosses were then tested and bred in various ways. Only one strain (line A) has been analyzed in detail, and conclusions, for the present, must be limited to the tailless condition as found in this strain. There are indications that the main features of the inheritance of taillessness are similar in the other strains.

The data for line A crosses are shown in table 2. Observations were all made as near birth as possible, usually within 12 hours of parturition. The distinction between the three classes, normal tail (nt), short tail (Brachy or Br), and tailless (zero tail or Ot) can be made at birth with little uncertainty. In the tailless animals there is no caudal skeleton at all, and the tail filament, which is nearly always present, hangs limply from the end of the sacrum; in short-tailed animals there is a stiff cartilaginous tail of from $\frac{1}{8}$ to $\frac{7}{8}$ of the length of the normal, ending bluntly, usually with a thin thread-like filament attached, the remnant of the resorbed posterior end of the embryonic tail; in normals the tail tapers to a point and never shows a filament.

Crosses of line A tailless and normal. The cross of tailless (line A) and normal (inbred Bagg albino) gives regularly two types of offspring, normal and Brachy. When the tailless parent is the mother, this segregation occurs in a 1:1 ratio (table 2, Exp. 1). When the tailless parent is the father, there is a considerable excess of normal offspring (Exp. 2), a difference which appears whenever taillessness is introduced through the male and which requires detailed discussion later.

The F_1 Brachy offspring behave like ordinary Brachy animals of the ZAWADSKAIA Brachy stock. When bred together or crossed with other Brachys, they produce only Brachys and normals (Exp. 10), and the homozygous Brachy type of lethal embryo is found in the uterus at the 10th day and dies shortly thereafter. When bred to normals, they produce Brachys and normals in a 1:1 ratio (Exp. 11). They do not transmit the tailless condition as found in the tailless parent line (11 females and 6 males thoroughly tested by Brachys from stock) and are evidently of the constitution $T/+$, like the usual Brachy animals. This shows that the A tailless parent is heterozygous for Brachyury.

TABLE 2

Results of matings of tailless mice of line A and of tests of their descendants.

EXP. NO.	PARENTS		GEN.	NO. OF MATINGS	TOTAL YOUNG	LITTER SIZE	OFFSPRING		
	♀	♂					NORMAL	TAILLESS	BRACHY
1	Ot	Normal*	F_1	20	114	5 7	63		51
K‡	Ot	Normal	F_1	5	25	5 0	12		13
2	Normal*	Ot	F_1	30	179	6.0	140		39
K‡	Normal	Ot	F_1	9	46	5.1	46		
3	Ot	Brachy	F_1	5	24	4.8	7	11	6
4	Brachy	Ot	F_1	37	304	8.2	128	127	49
5	F_1 nt (from 1)	Brachy	TC	47	335	7.1	164	87	84
6a	Brachy	F_1 nt (from 1 and 2)	TC	65	484	7.4	242	171	71
6b†	Brachy	F_1 nt (repetition)	TC	124	935	7 5	491	297	147
7	F_1 nt (fr 1)	Normal*	BC_1	22	144	6 6	144		
8	F_1 nt (fr 1)	F_1 nt (fr 1)	F_2	12	80	6.7	80		
9	F_1 nt (fr 1)	Ot (stock)	BC	19	89	4.7	53	22	14
10	F_1 Brachy (fr 1)	Brachy (stock)	TC	41	229	5.6	90		139
11	F_1 Brachy (fr 1)	Normal*	BC	10	53	5.3	33		20
12	F_1 Brachy	Ot (stock)	BC	8	52	6.5	21	27	4
13	BC_1 $+/t^{\circ}$ (fr 7)	Brachy	TC	34	200	5.8	90	55	55
14	Brachy	BC_1 $+/t^{\circ}$ (fr 7)	TC	20	169	8.5	85	47	38
15	BC_1 $+/+$ (fr 7)	Brachy	TC	42	295	7.2	149		146
16	Brachy	BC_1 $+/+$ (fr 7)	TC	10	91	9.1	51		40
17	BC_1 $+/t^{\circ}$ (fr 7)	Normal*	BC_2	7	45	6 4	45		
18	F_2 $+/t^{\circ}$ (fr 8)	Brachy	TC	20	127	6.4	57	37	33
19	Brachy	F_2 $+/t^{\circ}$ (fr 8)	TC	12	85	7.1	35	26	24
20	Brachy F_2 $+/+$ (fr 8)	F_2 $+/+$ (fr 8) Brachy	TC	22	158	7.2	79		79
21	$BC_2$$+/t^{\circ}$ (fr 17)	Brachy	TC	5	34		16	9	9
22	Brachy	BC_2 $+/t^{\circ}$ (fr 17)	TC	12	100	8.3	53	32	15
23	Brachy	BC_3 $+/t^{\circ}$	TC	25	222	8.9	118	77	27
24	$BC_3$$+/t^{\circ}$	Brachy	TC	9	53	5.9	27	14	12

‡ Data from KOBOZIEFF (1935).

* All normals were from our inbred stock of Bagg albinos.

† Offspring from two males omitted; cf. table 5.

The F_1 normal-tailed animals all transmit taillessness (31 males and 29 females thoroughly tested). This is best revealed by crossing F_1 normal females to Brachy males from stock. This produces about 2/4 normals, ¼ Brachys, and ¼ tailless (Exp. 5). Normals not derived from tailless produce, when crossed with Brachy, only Brachy and normal progeny. This shows that the tailless parent has transmitted a recessive gene for taillessness, which we may call t^0, to all normal-tailed F_1 progeny, thus proving the tailless parent to have been heterozygous both for T and for t^0. We may therefore assume that A tailless animals are T/t^0, F_1 Brachys $T/+$, and F_1 normals $t^0/+$. Taillessness is assumed to arise from interaction between T and t^0.

The constitution of the F_1 normals was also tested in several other ways. F_1 females were backcrossed to the tailless parent. This produced normal-tailed, Brachy and tailless progeny (Exp. 9).

F_1 normal females backcrossed to the normal parent stock produced only normal progeny (Exp. 7). These BC progeny, when tested by crossing, to Brachy, fell into two groups. One group (26 thoroughly tested) produced only Brachys and normals and were thus $+/+$ (Exps. 15, 16); the other group (39 thoroughly tested) produced normals, Brachys and tailless and were thus $+/t^0$ (Exps. 13, 14). The latter were again backcrossed to normal parent stock (Exp. 17), and the normal progeny so produced were again shown to consist of $+/+$ (7 tested) and $+/t^0$ (9 tested, Exps. 21, 22). The latter were again backcrossed to normal (Bagg) stock and the BC_3 progeny when tested fell into two groups: $+/t^0$ (Exps. 23 and 24) and $+/+$. By thus successively backcrossing normal animals, which were known to transmit taillessness, to the same inbred normal stock, any other genes affecting tail form tended to become homozygous, and the clear segregation of only two genetic types, $+/+$ and $+/t^0$, shows that a single gene, t^0, is involved.

F_1 normals bred together produced only normals (Exp. 8), but these F_2 normals, when tested by Brachys, fell into two groups, one of which (17 tested) produced tailless progeny in addition to normal and Brachy (Exps. 18, 19), while the other (9 tested) produced no tailless progeny (Exp. 20).

Thus the gene responsible for taillessness was shown to be transmitted through normal animals for four generations, to produce no effect on the tail when in combination with a normal allele, but to produce typical taillessness when again combined with T.

Crosses of line A tailless by Brachy. A direct test of the assumed constitution of the A tailless females was made by crossing them with Brachys from Brachy stock. If our asumptions are correct, this cross should have the following results:

A tailless × Brachy
T/t^0 $T/+$

T/T	T/t^0	$t^0/+$	$T/+$
dies	tailless	normal	Brachy

The expected classes were obtained, although, because of infertility of tailless females, the numbers are as yet too small to establish the ratio with certainty (Exp. 3). The reciprocal cross of tailless male made by

Brachy female yielded tailless, normal and Brachy progeny (Exp. 4, 12), as discussed below.

This may be taken as confirming our assumption that tailless animals are T/t^0. What then explains the fact that such hybrids, when bred together, produce only one type of offspring? Two assumptions need to be added: (1) that the homozygote t^0t^0 is inviable; (2) that T and t^0 are either alleles or else crossing over between them is prevented by close linkage or by some other means. Since all our data are consonant with the allele interpretation we shall use this until disproved. Matings of A tailless by A tailless would then be:

$$T/t^0 \times T/t^0$$

T/T	T/t^0 t^0/T	t^0/t^0
Brachy homozygote dies 10–11 days	tailless	tailless homozygote dies early

Litter size. If this is correct, litters from $T/t^0 \times T/t^0$ should be 50 percent smaller than litters from $T/t^0 \times +/+$, and should contain two groups of dead embryos: one, the Brachy homozygous type already studied; another, a new type due to the combination t^0t^0. Litter size may be influenced by factors other than the action of lethal genes (residual heredity of stocks, age of mother, ordinal number of litter, etc.), so that comparisons of litter size may not provide crucial evidence of the number of lethals segregating. In the present case, 66 liters from A tailless by A tailless contained 239 young, an average of 3.6 per litter. The average size of 50 litters from tailless by normal (Bagg albino) was 5.9. The former is 60 percent of the latter; it is smaller than the 75 per cent expected if only one lethal were segregating in tailless by tailless matings.

EVIDENCE FROM EXAMINATION OF EMBRYOS

Direct evidence of two lethals was obtained from dissections of the uteri of tailless females pregnant by tailless males (table 3). Examinations were made usually on the 11th day after timed copulations. Three types of embryo were found: (1) living embryos representing the viable tailless class; (2) abnormal embryos identical with the previously recognized and studied homozygous Brachy type (CHESLEY 1935); these were either still alive or had recently died; (3) embryos which had died shortly after implantation, consisting only of a small mass of resorbing material. These masses were of remarkably uniform size, indicating that the group as a whole had died at about the same time. The swellings of the uterus containing these masses were smaller than those containing normal or homozygous Brachy embryos. Of the resorbing masses dissected at the 11th day, each contained a well developed placenta with a completely resorbed embryo. Some uteri (tailless × tailless) were fixed entire on the 6th, 7th, and 8th days after copulation, and the embryos were then dissected out and sectioned. Preliminary examination of these shows that resorption of the abnormal type begins probably on the 6th and is certainly complete or nearly so by the 8th day. Eighth day dissections showed 39 normal, 24 resorbed and four swellings in which it could not be determined whether embryonic material had been present or not.

TABLE 3

Embryos found on dissection of uteri of pregnant females (from timed copulations).

MATING	MOTHER	FATHER	MOTHERS DISSECTED	EMBRYOS				VIABLE EMBRYOS PER LITTER
				VIABLE	BRACHY HOMOZYGOTES *TT*	RESORBED t^0t^0	?	
1	AA tailless	AA tailless	24	73	22	65	6	3.0
2	Normal (Bagg)	AA tailless	2	20		--	3	10.0
3	Brachy	AA tailless	14	114	12	3	9	8.1
4	*Normal $+/t^0$	Normal $+/t^0$	19	102	- -	58	8	5.4

* F_1, BC and F_2 animals from tailless by normal, which had been tested and found to be $+/t^0$

The resorbed group probably contains the t^0t^0 class, although it may contain also a few embryos which died after implantation from other causes. As controls, normal females pregnant by tailless males were dissected. Out of 23 embryos none of the resorbing type was found. In the case of three capsules no decision concerning the presence of embryonic tissue could be made. Likewise Brachy females pregnant by tailless males were dissected. About 10 per cent of the embryos were of the homozygous type, again proving that tailless males transmit brachyury. Three out of 138 embryos appeared to be of the resorbed type (table 3, mating 3), although these were not sectioned and the diagnosis was indicated as doubtful. Certainly there is no approach here to the frequency of the resorbed type found among tailless by tailless matings.

The data from dissections agree well with those from litters observed at birth. Thus in tailless by tailless matings the average litter size was 3.6 (table 1, Exp. 1); at the 11th day the average number of viable embryos was 3.0 per litter. The average number of embryos implanted was 6.9 of which 56.5 percent died after implantation as compared with 50 percent expected to die if two lethals are present.

F_1 normal-tailed females ($+/t^0$) from the cross to normal (Bagg albino) pregnant by their brothers ($+/t^0$), when dissected in the 10th day of pregnancy, showed 35.6 percent of resorbing embryos of similar size to those found in the uteri of tailless females (table 3, mating 4). This group probably contains the t^0t^0 lethal class expected, but the proportion is higher than the 25 percent expected. A few $+/t^0$ females from BC_1 and F_2 pregnant by $+/t^0$ males showed a similar proportion of the resorbing class.

ALLELISM OF T AND t^0

The results described above are consistent with the assumptions that the tailless line studied is heterozygous for the previously known dominant T, and for a recessive lethal t^0 which, when present with T, results in taillessness. These appear not to cross over. Crossing over would result in gametes without either gene which, when fertilized (in *inter se* tailless matings) by a T gamete, would result in Brachy offspring or, by t^0 gametes, would give normal offspring. Neither of these has been observed among 239 offspring of tailless×tailless in our experiments or among 63 offspring reported for the same line by KOBOZIEFF (1935). Likewise 60 F_1 normals from tailless by normal were shown to be $t^0/+$, indicating no crossing over in 60 gametes of tailless. It is probable then that T and t^0 are alleles and that these two lethals are balanced in the tailless line by their allelism. If this is so, then two alleles, each of which is lethal when homozygous, are not lethal when combined with each other (T/t^0), a situation which is sufficiently unique to be regarded with some suspicion as a final explanation. If it proves to be correct, then it will show that the dominant allele T is not a deficiency, since when combined with a lethal t^0 opposite to it, a viable embryo results. The non-lethal nature of the compound T/t^0 suggests that these alleles influence sufficiently different physiological processes so that a defect in one is made good by the other. The probable existence of another lethal allele in other tailless lines (see below) which in combination with t^0 leads to normal development points in the same direction. Further discussion is deferred until a more rigid test of allelism is obtained.

EXCESS OF TAILLESS OFFSPRING FROM t^0 FATHERS

The hypotheses proposed fit the facts obtained, when t^0 is introduced from the mother. When the father contains t^0, however, the proportions of offspring containing t^0 show significant departures in excess of the expected. Tailless females crossed with normal (Bagg) males produced 63 normals ($+/t^0$) and 51 Brachys ($+/T$); the reciprocal cross gave 140 normals and 39 Brachys. Normal females heterozygous for t^0 (from F_1, BC_1, BC_2 and BC_3), when crossed with Brachy males, gave the results shown in table 4 as compared with those expected on the above assumptions. Comparable matings of $+/t^0$ males by Brachy females gave the results in table 5.

TABLE 4

PARENTS			OFFSPRING	
		NORMAL $+/+, +/t^0$	TAILLESS T/t^0	BRACHY $T/+$
F_1 ♀ $+/t^0$×Brachy $+/T$♂		164	87	84
BC_1 ♀ $+/t^0$×Brachy $+/T$ ♂		90	55	55
BC_2 ♀ $+/t^0$×Brachy $+/T$ ♂		16	9	9
BC_3 ♀ $+/t^0$×Brachy $+/T$ ♂		27	14	12
F_2 ♀ $+/t^0$×Brachy $+/T$ ♂		57	37	33
Totals	actual	**354**	**202**	**193**
	expected 2:1:1 ratio	*374.5*	*187.2*	*187.2*
Sex ratios (per cent ♂♂)		47.2	58.0	50.9

TABLE 5

PARENTS		OFFSPRING	
	NORMAL	TAILLESS	BRACHY
F_1 ♂ $+/t^0$×Brachy ♀ (1st test)	242	171	71
F_1 ♂ $+/t^0$×Brachy ♀ (repetition) (six Type 1 males only)	491	297	147
BC_1 ♂ $+/t^0$×Brachy ♀	85	47	38
BC_2 ♂ $+/t^0$×Brachy ♀	53	32	15
F_2 ♂ $+/t^0$×Brachy ♀	35	26	24
BC_3♂×Brachy ♀	118	77	27
Total	**1024**	**650**	**322**
Expected 3:2:1 ratio	*998*	*665.3*	*332.6*
Sex ratios (per cent ♂♂)	45.8	54.9	48.7
F_1 ♂ $+/t^0$×Brachy ♀ (two type 2 males only)	159	124	34
Sex ratios (per cent ♂♂)	46.5	56.5	45.6

These results clearly establish that $+/t^0$ males produce a significantly higher proportion of tailless progeny when tested by Brachy than do $+/t^0$ females. The $+/t^0$ males in table 5 are sibs of the $+/t^0$ females in table 6 and the Brachy animals used were all from the same Brachy stock.

Moreover, different $+/t^0$ males appeared to give different proportions of tailless progeny. A special test of this point is in progress (repetition of F_1 ♂×Brachy, table 5). Eight F_1 $+/t^0$ males have been tested by crossing with Brachy females. Six of these have each produced normal, tailless and Brachy progeny in a ratio approximating 3/6 normal; 2/6 tailless; 1/6 Brachy (actually 491:297:147). Two males each gave very few Brachy offspring (actually 159 normal:124 tailless:34 Brachy). The existence of two types of $+/t^0$ males indicates that another factor or factors are involved and these are being studied further. The difference is not due to the source of the t^0 gene since males receiving it from the mother produce progeny in the same proportions as those receiving it from the father.

Omitting the results from males shown to be of the second type, the ratios as a whole give a good fit to a 3:2:1 ratio.

The departure of this from the 2:1:1 ratios regularly obtained from $+/t^0$ females is probably not due to sex-linkage. The t^0 gene is certainly not sex-linked, since it is transmitted regularly to both sexes by T/t^0 and by $+/t^0$ males and females. No marked variations in the sex ratios of the three types of progeny obtained from crosses of these genotypes by Brachy females have been observed (tables 4 and 5). The slight excess of males among the tailless progeny may be due to differential viability.

The peculiarity in inheritance of t^0 is its more frequent transmission through the sperm than through the egg. Most males of the type $+/t^0$ appear to produce about twice as many t^0 as + sperm, as inferred from the production of twice as many T/t^0 as $T/+$ progeny when crossed with Brachy. Such a condition might arise if t^0 spermatocytes were to undergo one further equational division although we have no direct evidence on this.

A similar excess of offspring with t^0 is found wherever t^0 is introduced from the male. Thus tailless males by Brachy females gave 121 normals ($+/t^0$), 46 Brachy ($+/T$) and 122 tailless (T/t^0); the tailless progeny outnumber the Brachys by over two to one, equality expected. However, normal (Bagg albino) females by tailless males gave 140 normal ($+/t^0$) and only 39 Brachys ($+/T$), a wide departure from a 2:1 ratio. Here again individual tailless males may give different ratios and this must be tested in new experiments. KOBOZIEFF (1935) also crossed normal females from a laboratory stock of albinos with A tailless males and obtained 46 *normals* only. His reciprocal cross gave 12 normals and 13 Brachys; ours gave a similar result, 63 normals and 51 Brachys. KOBOZIEFF attributed the difference to genetic differences in the normal animals used in the reciprocal crosses; but this cannot be true in our case, since we have used only animals from the 40th–50th brother-sister generations of our stock of Bagg albinos. The differences are probably due to peculiarities in the transmission of t^0 by males.

The ratios of non-viable embryos reveal a similar distortion whenever t^0 is introduced through the male. Thus dissections of tailless females pregnant by tailless males gave unexpected ratios as follows:

T/t^0 ♀ × T/t^0 ♂	FOUND PERCENT	EXPECTED (NORMAL SEGREGATION)	EXPECTED (2 t^0:1 T SPERM)
Brachy homozygotes TT (dead)	13.8	25	16.6
Early resorption t^0t^0 (dead)	40.6	25	33.3
Viable T/t^0	45.6	50	50

The data do not fit the ratio expected if both eggs and sperm segregate in 1 T:1 t^0 ratios; the fit is better, although still is not perfect to the assumption of a 1:1 ratio among the eggs but a 2 t^0:1 T ratio among the sperm.

Similarly, in crosses of tailless male (T/t^0) by Brachy female ($T/+$) 25 percent of TT embryos are expected. This class is readily recognized and the classification is accurate. Actually only 10 percent were found in this cross, which correlates fairly well with the deficiency of Brachys ($+/T$) and the excess of tailless (T/t^0) among the progeny born (table 2, Exp. 4). Finally the dissections of matings of $+/t^0 \times +/t^0$ (F_1 normals from tailless×normal) showed 35.6 percent of the early lethal type t^0t^0, which is to be compared with 25 percent expected if segregation is normal in both sexes or with 33.3 percent if sperms segregate in 2 t^0/1+ ratio. Because of wide variations in different individual matings, this question will require further study.

TAILLESSNESS IN OTHER LINES

It has not yet been possible to complete a comparable analysis of the inheritance of taillessness in lines 29 and 19. Preliminary evidence however is sufficient to show that each of these lines is heterozygous for T and for some other recessive condition like t^0 which, in combination with T, produces taillessness. The additional gene in line 29 is probably not the same as that which occurs in line A, since these two lines when crossed produce both tailless and normal progeny (table 1). In our cross these occurred in a ratio of about 2:1. This would result, if line 29 carried an allele such as t^1 of the t^0 gene of line A. The normal progeny from the cross 29×A would thus be t^0/t^1, that is, the compound of two lethals would be viable. This hypothesis is being tested. Line 19 apparently differs from line A, but there is as yet no evidence (except mode of origin) to indicate that line 19 differs from line 29. No embryological evidence has yet been obtained regarding the assumed lethals in either of these lines.

SUMMARY

Experiments with a pure breeding line of tailless mice discovered by DOBROVOLSKAIA-ZAWADSKAIA show that such tailless mice contain two factors, T and t^0, which are probably alleles, each of which is lethal when homozygous. The combinations of these have the following effects:

Tt^0—tailless;

TT—characteristically abnormal embryos dying about 11 days after fertilization;

t^0t^0—abnormal embryos dying shortly after implantation, probably during the 7th day after fertilization;

$T+$—short-tailed (Brachyury);

t^0+—normal.

The segregation of t^0 from either T or $+$ occurs normally in oögenesis. When t^0 is transmitted by the male, a marked excess of offspring with t^0 is found, corresponding to a sperm segregation ratio of about $2\ t^0{:}1\ +$.

Taillessness probably rests on a similar basis in two other tailless lines, that is, the Brachyury lethal T may be balanced by a recessive lethal allele. The second lethal may be different in different tailless lines.

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AN ANALYSIS OF CHROMOSOME STRUCTURE AND BEHAVIOR WITH THE AID OF X-RAY INDUCED REARRANGEMENTS

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THE structure of the chromosome during various phases of the cell cycle is associated with coiling and twisting of the chromonemata. At mitotic anaphase the two chromatids of each chromosome are independently coiled, and remain coiled during the resting stage. At early prophase the minor coils relax and begin to expand. As the relic coils begin to straighten out, new minor coils form in each chromatid. During the prophase stages the two chromatids are twisted about each other or "relationally coiled." Contraction of the chromatids tends to eliminate the relational coiling, so few twists remain at metaphase, and the daughter chromosomes separate freely at anaphase.

The characteristic features of chromosome structure and behavior at meiosis include: (1) the elimination of relic coils *before* new minor coiling is initiated; (2) pairing and relational coiling of homologous chromosomes; and (3) the formation of major coils. The elimination of the relic coils before new minor coiling begins seems to be necessary as a preliminary to chromosome pairing (SAX and SAX 1935). The formation of major coils is not related to chiasma formation or the elimination of relational coiling of chromatids (O'MARA, unpublished). The relational coiling of chromosomes, and perhaps the relational coiling of chromatids in each homologue, seems to be involved in chiasma formation and crossing over (DARLINGTON 1935).

To arrive at an understanding of the mechanism of crossing over, it is essential that as clear a picture as possible be had of each detail of chromosome structure and behavior which is even remotely related to the process of chiasma formation. The relational coiling of chromatids in Tradescantia has been studied directly at second meiotic anaphase, late prophase, and metaphase of the microspore mitotic cycle, when the chromatids are easily seen, and indirectly at the interphase and early prophase by means of chromosome configurations which do not permit free separation of daughter chromosomes. It has been possible to demonstrate what appears to be an increase in the amount of relational coiling during the phase of the mitotic cycle when individual chromatids cannot be completely resolved. The chromosome configurations which make this demonstration possible are of further interest and will be described in some detail.

Microspores of *Tradescantia paludosa* were subjected to approximately 1,000 *r*-units of continuous X-radiation. The normal development of the microspores in this plant has been described in detail by SAX and EDMONDS (1933). The development from the tetrad stage to the division of the microspore nucleus requires about five days during the summer months. The nucleus appears to be in the resting stage for two or three days. During this period the microspore enlarges, and the orientation of the nucleus and cytoplasm is accomplished. The prophase stage begins on the third or fourth day, and the metaphase occurs on the fifth day. The duration of the cycle depends, to some extent, on weather conditions.

The X-ray treatment seems to be more effective in producing abnormality during meiosis than during the somatic resting stage. This differential effect provides a method for timing the duration of microspore development. The young microspores subjected to the X-ray dosage employed continued to develop. The microspores produced *after* X-ray treatment—that is, those which resulted from irradiated meiotic cells -were shriveled and sterile. The microspores which divided on successive days after treatment were X-rayed during the developmental period. When only aborted microspores appeared, it could be concluded that the microspore cycle had ended. The period from treatment to the appearance of only sterile microspores was from 8–9 days, as compared with 5 days for normal plants grown during the summer months. Part of this discrepancy is attributed to greenhouse conditions during the fall and winter months, and part to a retardation of development as a result of treatment.

On the day the treatments were given, and to a lesser degree on the day after, few satisfactory preparations were obtained. The chromosomes were badly clumped and poorly stained. On the second and subsequent days after treatment excellent preparations were possible. Since the cycle from the quartet stage to metaphase was completed in 8–9 days instead of 5, nuclei at metaphase 4 days after treatment were probably rayed during the early prophase stage. Those at metaphase 5–9 days after exposure were undoubtedly resting nuclei at the time the treatment was given. Acetocarmine preparations of the first microspore mitosis, smeared each day after treatment, were made permanent after the method described by BUCK (1935).

OBSERVATIONS

Before the anaphase chromosomes of the second meiotic division enter into the quartet resting stage, they are clearly coiled in the form of minor spirals. It is difficult to determine directly whether the chromosomes at this stage are made up of two spirally wound chromatids "which are not twisted around each other but run parallel as two independent spirals quite free from each other . . ." as KUWADA and NAKAMURA (1935) report. SAX and SAX (1935) have observed constricted regions in the chromosomes which they interpret as twists in two partially separated spirals, confirming the double nature of the second anaphase chromosomes reported by NEBEL (1932) and KUWADA and NAKAMURA (1935). An analysis of 50 second anaphase chromosomes revealed no constricted regions (half turns which spiralled chromatids make around each other) in 12 chromosomes, 1 constriction in 20 chromosomes, 2 constrictions in 16 chromosomes, and 3 in 2 chromosomes. In 9 of the chromosomes containing 2 constrictions, 1 occurred in each chromosome arm. In the chromosomes with 3 constrictions, 2 were found in 1 arm and 1 in the other. Some of the half turns observed may be cancelled by another half turn in an opposite direction in the same chromosome. Notwithstanding this possibility, a total of 58 half turns were found, or an average of 1.16 half turns per chromosome at second anaphase.[1]

At the earliest prophase, chromosomes are in the form of minor spirals. The minor spirals appear somewhat relaxed, but it is clear that they have not been eliminated during the resting stage. A similar observation has been reported by DARLINGTON (1935), who says: "The correspondence of the spirals seen at early prophase with those seen at telophase shows that no great change of position has taken place during the resting stage— that it is mechanically what its name implies." As prophase advances, the old minor, or relic spirals, appear to relax more completely. They are optically single. The gyres of these spirals tend to increase in diameter and decrease in number as the chromosome shortens. When the presence of relic spirals is no longer obvious, each chromosome is definitely made up of two chromatids which are independently spiralled and tend to be relationally coiled. At least 3 half turns of the chromatids around each other can be seen in some chromosomes at this stage (SAX and SAX 1935).

The direction of the relational coiling in 118 chromosome arms at late prophase and early metaphase was "right" in 60 arms and "left" in 58. The direction in both arms of 50 chromosomes was "right" in 18, "left" in 18; and in 14 chromosomes "right" in one arm and "left" in the other. The direction of relational coiling is apparently not at random in the 2 arms. In approximately a third, instead of a half of the chromosomes, the direction is reversed at the fiber attachment. At late metaphase the majority of the chromatids are parallel or, at most, make an occasional half turn around each other. The relational coiling is evidently reduced as the chromosomes shorten during prophase. In 195 chromosome arms at late metaphase, 56 half turns (or overlaps) were found. This is an average of 0.57 of a half turn per chromosome. The daughter chromosomes (3 with median and 3 with submedian fiber attachments) measure ca 10–12μ long and 1.3μ in diameter. They separate freely at anaphase.

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[1] I am indebted to Professor KARL SAX for the loan of the preparation from which these data were taken.

At the first mitosis after X-ray treatment, daughter chromosomes in three chromosome configurations do not separate regularly in the normal manner. Chromosomes with two attachments, chromosomes with fused chromatids, and ring-shaped chromosomes are configurations of this type. They differ from structurally unchanged chromosomes by usually breaking at the anaphase stage.

Chromosomes with two fiber attachments

Chromosomes with two fiber attachments were found frequently at metaphase of the microspore division. The chromatids between the two attachments were parallel or often twisted about each other. The daughter chromosomes were observed to disjoin in three ways at anaphase.

(1) Both of the attachments on each daughter chromosome go to the same pole. The chromosomes remain intact, and two daughter nuclei result, with the same chromosome situation as the parent nucleus (figs. 1 and 2).

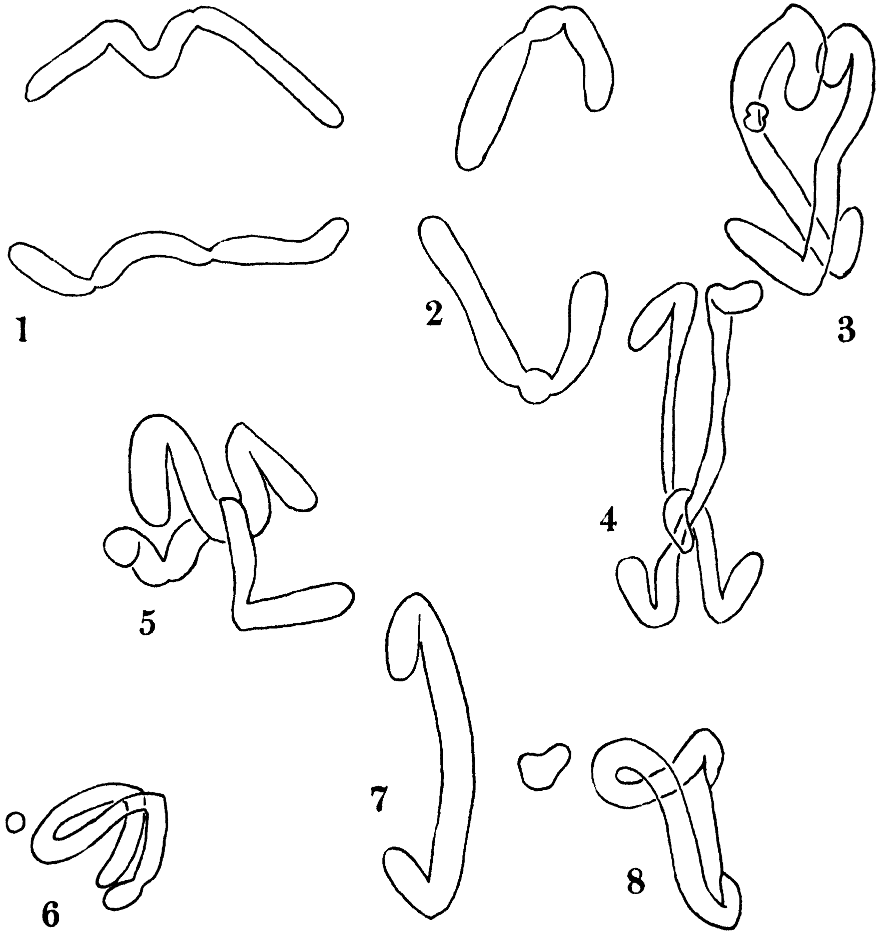
(2) The two attachments on each daughter chromosome go to opposite poles. The chromosomes then become attenuated and are observed to break, as they eventually must if disjunction is to be completed (figs. 3 and 4).

(3) The two attachments on each daughter chromosome go to the same pole but, because the chromosomes are interlocked, at least one and sometimes both chromosomes are broken (fig. 5).

In the cases where the two attachment points on each daughter chromosome pass to the same pole and the daughter chromosomes are interlocked (fig. 5), the two chromatids comprising the segments between attachments have made one complete turn about each other. In figure 3, one half turn is involved; in figure 4, three half turns. In figures 1 and 2, there is no twisting, and the two chromatids must have been lying parallel, in one plane at metaphase.

The number of turns two segments can make around each other is dependent upon the length of the segments and their diameters. If the amount of twisting per unit of chromosome length in one species is to be compared with the amount of twisting in a species with dissimilar chromosomes, both the length and diameter of the chromatids must be considered. The length of the segment between two attachments, divided by the diameter of the daughter chromosome, will give a ratio which can be used to express segment length and to compare twisting.[2]

The ratio was determined for twenty-five chromosomes which disjoin without breaking, for twenty-five which separate as in (2), above, and for twenty-five which separate in a way that necessitates the breakage of at least one daughter chromosome. The averages of these ratios and the extremes in each case are given in table 1. An anaphase chromosome with fiber attachments moving toward opposite poles appears approximately twice as frequently as a chromosome which is interlocked.



TEXT FIGURES 1–8. Mitotic anaphase chromosomes with two fiber attachments and chromosomes with fused chromatids. Figs. 1, 2, and 5. Two attachments on each daughter chromosome moving to the same pole. Figs. 3 and 4. Two attachments on each daughter moving to opposite poles. Fig. 6. Metaphase chromosome with fused chromatids. Figs. 7 and 8. Anaphase chromosomes with fused chromatids. X2360.

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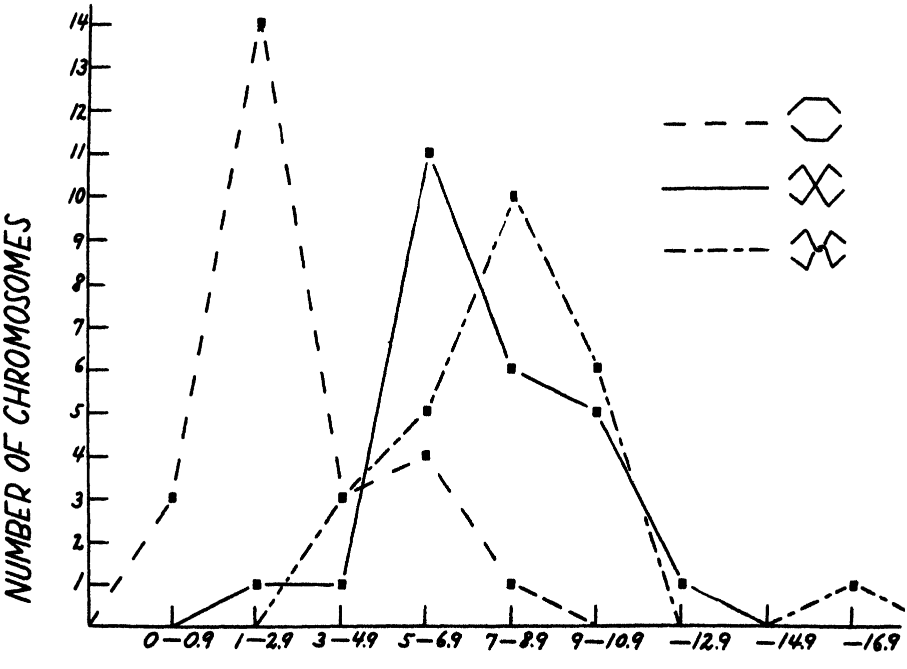
[3] Unless otherwise stated, the actual length divided by the average diameter of a daughter chromosome and the actual circumference divided by the average diameter of a chromatid or daughter chromosome will be referred to as "length" and "circumference," respectively.

The chromosomes which separate without breaking (1) have an average ratio of 2.5; those which separate in the manner which demands the breakage of both daughter chromosomes (2) have an average of 6.9; and those which have a breakage of at least one daughter necessary for disjunction (3) an average ratio of 7.5. The results show that at the first division after the formation of a chromosome with two fiber attachments, variation in its behavior can be expected depending upon the "length" of the chromosome segment between the two attachments. If the attachments are relatively close together, they behave usually as a unit, although one may apparently precede the other toward the pole. A chromosome with two attachments widely separated rarely disjoins completely without the breakage of one or both daughters. Seventeen of the 18 chromosomes

TABLE 1

Average "length" of segment between attachments.

Type of disjunction (see text)	(1)	(2)	(3)
Average "length" of segment between attachments	2.5	6.9	7.5
Extremes	0.7–7.0	2.0-11.0	3.0-16
Average "length" of segment in (2) and (3) per 1 full turn		9.6	
Number of chromosomes	25	25	25



TEXT FIGURE 9. Relation of "length" of segment between two attachments to type of separation at mitotic anaphase.

which have a ratio under 3 are separating without breaking. Twenty-nine of the 30 chromosomes which have a ratio of 7 or higher are separating in a manner which will result in the breakage of one or both daughter chromosomes (fig. 9).

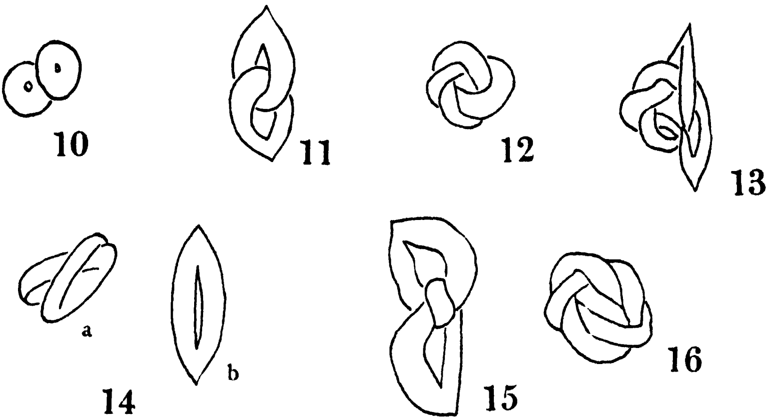
The three types of disjunction observed can most easily be interpreted as the result of twists present in the sister chromatids when the structural change, resulting in a chromosome with two attachments, was induced. In a normal chromosome these twists tend to straighten out during the contraction of the prophase chromosome, and few are present at metaphase. If chromatids do not rotate at the attachment point, the twists, or turns chromatids make around each other, present between the two attachments of a structurally changed chromosome, could not straighten out. When no turns exist between the attachments, the two attachments on each daughter chromosome pass to the same pole. Should a half turn, one and a half turns, etc., be present, the two attachments on each daughter move to opposite poles. A full turn, two, three turns, etc., necessitate the passage of the two attachments on each daughter to the same pole, and breakage results.

Fused Chromatids

"Chromatin bridges" have been frequently reported as a result of X-ray treatment. These extend from one anaphase group of chromosomes along the long axis of the spindle to the other pole. At the first metaphase after X-ray treatment, chromosomes are often seen which show only two free chromatid ends instead of four. The chromatids in one arm of these chromosomes are so completely fused that it is impossible to determine exactly where a union of ends has occurred (fig. 6). In every cell containing a chromosome with fused chromatids, chromosome fragments are also found. The fused chromatids do not allow free separation of the daughter chromosomes at anaphase. As a result, a chromatin bridge is formed (fig. 7). The attached sister chromatids become attenuated and undoubtedly break. Ring-shaped chromosomes are also found separating at anaphase. The lengths of the two chromatin segments between the sister attachments are of unequal length (fig. 8). These are most probably the result of two chromatid fusions. If the two chromatids of a chromosome with fused chromatids again fuse, after a breakage has occurred at anaphase, and subsequent breakages should occur at points other than at the point of previous union, gene duplication and deficiency would result in this type of chromosome as well as in a chromosome with two attachments.

Ring-Shaped Chromosomes

Sixty-five ring-shaped chromosomes were found at late prophase, metaphase, and anaphase of the first division after treatment. Ten of the 65 were clearly "disjunctional." The two chromatids comprising these rings were not twisted about each other (fig. 10). At anaphase the daughter chromosomes should separate as freely as the chromosomes with two attachments shown in figures 1 and 2. The "disjunctional" rings had an average "circumference" at metaphase of 3.8, the largest a "circumference" of 5. The average "inter-attachment" segment "length" in "disjunctional" chromosomes with 2 attachments (table 1) was nearly 3. Disjunctional rings may be formed by the union of one broken end of each chromatid with its other broken end.



TEXT FIGURES 10–16. Ring-shaped chromosomes at mitotic metaphase and anaphase. Fig. 10. "Disjunctional." Figs. 11–13. "Interlocked" ring-shaped chromosomes. Chromatids make one turn about each other in 11, two turns in 12, and three turns in 13. Figs. 14–16 "Continuous" ring-shaped chromosomes Chromatids make a half turn about each other in 14 (a, metaphase; b, anaphase), one and a half turns in 15, and two and a half turns in 16. X2360.

Thirty-seven of the ring-shaped chromosomes were "interlocked." The two chromatids in 31 of them made one complete turn around each other (fig. 11). Twenty-eight of these 31 rings were at metaphase or anaphase, had a circumference no greater than the length of a normal metaphase chromosome, and an average "circumference" of 7.5. The chromosomes with 2 attachments in which the chromatids make one full turn around each other (fig. 5) had an average "inter-attachment" segment "length" of 7.5. In 5-ring chromosomes, the chromatids made 2 full turns around each other (fig. 12), and in one chromosome 3 full turns (fig. 13). The chromosome in figure 13 is at anaphase. It is somewhat stretched and measures ca 16μ in circumference. At metaphase it probably had a circumference no greater than the length of a normal chromosome. These ring-shaped chromosomes range from 3μ in diameter to 16μ in circumference. They may be formed by the union of one broken end of each chromatid with its other broken end, the sister chromatids being twisted 1–3 times around each other prior to the union.

Eighteen of the ring-shaped chromosomes were "continuous." In 14 of them, the two chromatids made a half turn around each other (fig. 14). The 10 simple "continuous" rings which were at metaphase or anaphase, could be measured and had a circumference no greater than the length of a normal chromosome, had an average "circumference" of 6.5. The chromosomes with two attachments in which the chromatids made a half turn around each other had an average "inter-attachment" segment "length" of 6.9. In 3 of the ring chromosomes the chromatids made 1.5 turns around each other (fig. 15); and in one, 2.5 (fig. 16). The "continuous" rings may be formed by the union of one broken end of each chromatid with the other broken end *of its sister*, 0–2 *additional* full turns of the sister chromatids about each other being involved. The 38 simple "interlocked" and "continuous" ring chromosomes measured had an average "circumference" of 8.3 per full turn.

At anaphase the "interlocked" rings cannot disjoin without the breakage of at least one daughter chromosome. The simple "continuous" ring opens out after metaphase to form a chromosome twice the size of the original configuration. This double-sized ring, as well as the more complex "continuous" rings, must break in two places during the anaphase separation.

There does not appear to be a statistically significant difference in the average "circumference" of the simple "interlocked" and "continuous" ring-shaped chromosomes produced by X-ray treatment of the early resting, the late resting, or the early prophase stages (table 2). Five of the more complex ring-shaped chromosomes (that is, those in which the

TABLE 2

Average "circumference" of ring-shaped chromosomes.

Number of days between x-ray treatment and metaphase	2-4	5	6–9	2–9
Approximate stage treated	early prophase	late resting	resting	—
Average "circumference" of simple interlocked rings	7.3±1.01*	6.98±.49	8.64±.75	7.47
Number of chromosomes	7	14	7	28
Average "circumference" of simple continuous rings	—	6.6±1.12	6.46±1.16	6.53
Number of chromosomes	—	5	5	10
Average "circumference" of simple continuous and interlocked rings	7.3	6.87	7.73	7.23
Total number of chromosomes	7	19	12	38

* $s^2 = \frac{\Sigma(x^2)}{N-1}$; $S_{\bar{x}} = \frac{s}{\sqrt{N}}$

chromatids make more than 1 full turn around each other) resulted from treatment applied at the resting stage, and five from treatment given at early prophase.

DISCUSSION

A normal metaphase chromosome at the first microspore mitosis in Tradescantia has a "length" of ca 4–5 for each arm. The chromatids of this chromosome are usually lying parallel, or at most make a half turn around each other. An average of ca 2 half turns for every 7 arms, or 0.57 of a half turn per chromosome, is present at this stage. In figure 9, 7 half turns are found between the 2 attachments in the 7 segments with "lengths" of 3–4.9. The 50 chromosomes with 2 attachments, between which ½ or 1 full turn occurs, have an average "length" per 1 full turn of 9.6 (table 1). The 38 ring-shaped chromosomes, in which ½ or 1 full turn of the chromatids around each other is present, have an average "circumference" per 1 full turn of 8.3. The number of turns per unit of "length" and "circumference" in chromosomes with 2 attachments and ring-shaped chromosomes is approximately equal and over three times the average number of turns found in a normal metaphase chromosome.

There may be at least 1.5 full turns of the chromatids around each other in a normal chromosome at late prophase. At metaphase this number has been reduced, so at most an occasional half turn or overlap is present. A reduction in the amount of relational coiling is obviously prevented in ring-shaped chromosomes and chromosomes with two attachments. In ring-shaped chromosomes there are no free ends to rotate. Any relational coiling present at the time these chromosomes were formed could not be eliminated by the contraction of chromosomes during prophase and early metaphase. Since the amount of relational coiling in ring-shaped chromosomes and chromosomes with two attachments is approximately equal at metaphase, an interesting property of the fiber attachment is demonstrated by means of these configurations.

The fiber attachment has been known for some time as the constant and well differentiated portion of a chromosome which separates first at anaphase and precedes the body of the chromosome toward the pole. In many organisms the chromatids are so intimately associated at the fiber attachment that this limited section of the chromosome appears to be single. At late metaphase, after the chromatids have become oriented, the anaphase separation is first initiated at the region of the chromosome which previously showed no evidence of being two-parted. The character of the fiber attachment region apparently changes abruptly during metaphase. The change, according to some workers, is probably brought about by its division at this stage. The fiber attachment is known to be four-parted at metaphase of the microspore division, and two-parted at anaphase in chromosomes which are especially favorable material for the study of chromosome structure (Trillium; HUSKINS and HUNTER 1935). A half turn of sister chromatids around each other may be present in a segment between two attachments which is no longer than twice the diameter of a daughter chromosome (fig. 9). The contraction of the chromosome does not produce a strain which is great enough to cause a rotation of the chromatids at the fiber attachment. Although this portion of the chromosome is 2-parted, it behaves essentially like an undivided region prior to anaphase.

The direction of relational coiling in the arms of a Tradescantia chromosome does not appear to be at random. It changes at the fiber attachment in only about a third of the chromosomes. If, in the formation of a ring-shaped chromosome, breakage occurs at the time of treatment, and the union of broken ends is accomplished immediately, there would be no opportunity for an untwisting of chromatids that are relationally coiled in the same direction on both sides of the attachment, between the time of treatment and metaphase. The average number of turns sister chromatids make around each other per unit of chromosome "circumference" at metaphase would be a rough index of the number present at the time of treatment. If, on the other hand, breakage occurs at the time of treatment and union is delayed, the amount of relational coiling in ring-shaped chromosomes would be an indication of the average amount present when the union of broken ends is accomplished. This union may not take place until chromosome contraction at prophase has eliminated some of the relational coiling. The chromatids of the ring-shaped chromosomes (and chromosomes with two attachments) average 2 half turns around each other in a "circumference" (or "length") which is slightly less than the "length" of a normal metaphase chromosome. It seems evident that the chromatids of such chromosomes averaged at least 2 half turns around each other at the time the broken ends united. In one ring-shaped chromosome 6 half turns were found. Approximately a third of the ring-shaped chromosomes were continuous, and no "disjunctional" ring had a "circumference" greater than half the "length" of a normal chromosome.

The two chromatids of a second anaphase chromosome in Tradescantia may average 1.16 half turns around each other (maximum number of half turns found in one chromosome is 3. After the formation of a ring-shaped chromosome, a half turn of chromatids around each other on one side of the attachments would be cancelled by a half turn in the opposite direction on the other side. When it is considered that the direction of relational coiling in the two arms of a second anaphase chromosome may be at random, or may change at the fiber attachment in about a third of the chromosomes (as it does at prophase), the average amount of relational coiling at anaphase, which would be expected to persist in a ring-shaped chromosome, becomes nearer one half turn per chromosome. The ring-shaped chromosomes (as well as the chromosomes with two attachments) seem to indicate that the amount of relational coiling is increased to an average of at least two half turns per chromosome (maximum number of half turns in one ring-shaped chromosome is 6) some time before late prophase of the next mitotic division. The maximum amount of relational coiling seen in the ring-shaped chromosomes is twice the maximum amount in a normal chromosome at the preceding anaphase. In structurally unchanged chromosomes, the average amount is reduced, as a result of chromosome contraction during prophase to 0.57 of a half turn which remains at metaphase.

McClintock (1932) has shown that broken ends of chromosomes have a strong tendency to unite with broken ends. Whether the union immediately follows breakage or is possibly delayed, even for some cell generations, has not been definitely determined (Stadler 1932). The similarity of the results of X-ray treatment applied during the early resting, late resting, and early prophase stages may be brought about by the delay of the union of broken ends until one prophase period, regardless of the stage at which the chromosomes were broken, or by a union concomitant with breakage. If the union of broken ends immediately follows breakage, an increase in the relational coiling of chromatids must occur during the telophase which precedes the resting and early prophase stages exposed to X-rays. If the chromosome ends, broken at different periods of the resting and early prophase stages, unite at a given midprophase period, an increase in relational coiling could take place at the beginning of prophase rather than at the preceding telophase.

A brief consideration of mitosis and meiosis may suggest which of these explanations is the more tenable, and at what stage of the somatic cell cycle an increase in relational coiling might be brought about. The two chromatids of a mitotic anaphase chromosome seem to be in the form of tightly intermeshed (not interlocked) minor spirals (Nebel 1932; Kuwada and Nakamura 1935). They do not appear to be twisted around each other to any great extent. During the resting stage the spirals become relaxed, but they are not eliminated (Darlington 1935; Sax and Sax 1935). As the nucleus enters mitotic prophase and enlarges, the gyres of each relic spiral become wider, and their number seems to be reduced as prophase advances and the chromosomes become shortened. When the spirals are nearly straightened out, each chromatid is seen to be independently spiralled, and tends to be relationally coiled. The new spirals further shorten the chromatids during later prophase. The twists are passed off the ends of the chromosome, so few remain at metaphase.

The increase in size of the early meiotic nucleus and the leisurely progress of the meiotic prophase permit a complete removal of the relic spirals before the new spiralization begins. At pachytene the chromosomes are extended to approximately twice the length seen at a comparable mitotic prophase (SAX and SAX 1935). The mitotic and premeiotic resting stages appear to be identical. The essential difference between a mitotic prophase and an early meiotic prophase is the period at which the new spiralization begins in relation to relic spiral elimination. In mitotic prophase spiralization of chromatids begins at early prophase, before the relic spirals of anaphase are drawn out. When the relic spirals of the somatic chromosome are eliminated, the new minor spirals are well formed and the chromosome has been shortened to half its expanded length as seen in a meiotic chromosome at pachytene. Relational coiling of chromatids is easily reduced as a chromosome shortens during late mitotic prophase, and becomes more rigid as the formation of minor spirals is completed. In meiotic prophase the chromosome elongates and "pairs" with its homologous chromosome. The elimination of the relic spirals (which is probably the major factor in the mechanism of elongation) is not accompanied by the formation of new minor spirals. The formation of these spirals is not initiated until *after* homologous chromosomes have become intimately "paired" (SAX and SAX 1935).

A modification of the torsion theory of crossing over, proposed by WILSON and MORGAN (1920), seems to be most in accord with the cytological observation of bivalent chromosomes. This theory demands that the chromatids in each chromosome which associates with its homologue be parallel and *not twisted around each other* (SAX 1936). If this theory is sound, and if in nature there is an actual increase in the amount of relational coiling of chromatids at the telophase stage of mitosis, this coiling would have to be eliminated before the association of homologous chromosomes at meiotic prophase occurs. At early meiotic prophase the chromosomes are elongating and presumably becoming more flaccid. These chromosomes would more probably retain any relational coiling present than remove it. If an increase in the amount of relational coiling actually occurs when new minor spirals are being formed (that is, at early prophase of mitosis and late pachytene of meiosis) the requirement of the torsion theory, in regard to relational coiling of chromatids, could be fulfilled. It seems more probable at present that an increase in relational coiling of chromatids is initiated at early prophase in mitosis when the formation of new minor spirals begins; and that the union of ends broken at the resting and early prophase stages is not definitely accomplished until a later prophase period.

How should the chromosomes with two attachments and the ring-shaped chromosomes described at the first mitosis after their formation be expected to behave during later somatic divisions? MATHER and STONE (1933) have reported the occurrence of chromosomes with two attachments in X-rayed Crocus and Tulipa root tips. They point out two ways in which these chromosomes can separate: the two attachments on one daughter chromosome can go to the same or to opposite poles. If they move to opposite poles, the chromosomes become attenuated and probably break, eventually giving rise to daughter cells which have chromosomes with only one attachment and which differ from the parent nucleus. It is reasoned, since the chances of either mode of division are taken to be equal, that the probability of a chromosome with two fiber attachments surviving after n cell generations is 2^{-n}; that "there is . . . a strong tendency for such chromosomes to disappear. . . ." This seemed to be the case in Crocus. Fixations made soon after treatment showed "a few" chromosomes with two attachments, while later fixations showed none at all. In Tulipa, however, four chromosomes with two attachments (two of the four existing together in a single nucleus) were found in root tips fixed three months after treatment.

These chromosomes, illustrated in MATHER and STONE'S figure 32, have ratios of ca 5, 11, 13, and 14. If the original chromosomes from which these four descended had like ratios, and it is assumed that Tulipa chromosomes with two attachments behave, at the first division after their formation, like Tradescantia chromosomes, only one of the four could have disjoined without a breakage.

If fifty can be taken as a fair estimate of the number of cell generations accomplished during a period of three months and the chances of either mode of division of a chromosome with two attachments are equal, the probability of such a chromosome surviving after fifty cell generations would be 2^{-50}. The finding of four of these chromosomes suggests that the chances of either mode of division are unequal, or, when breakage occurs, the broken ends tend to unite like the broken ends of ring-shaped chromosomes.

MCCLINTOCK (1932) and MCCLINTOCK and RHOADES (1935) have presented the most complete study of ring-shaped chromosomes and their behavior. These chromosomes do not descend through many cell generations unaltered. Losses, and less frequently additions, of sections of chromatin are continually occurring. These changes, they have suggested, may be causally related to the way a ring chromosome becomes split during mitosis. The product of chromosome splitting is not always two ring chromosomes lying side by side. "Instead, especially with large rings, many anaphase figures reveal that the end product of the splitting process has produced two interlocking rings or one large double-sized ring with two spindle fiber attachment regions. In this latter case, the large single ring with the two spindle fiber attachment regions is built up from both split halves of the mother ring. On the simple hypothesis that the chromosome may start its splitting process at more than one place in the chromosome and that the planes of the splits so started do not correspond, it is relatively easy to see how such figures could be obtained. In both the interlocking and double-sized rings, the pull on the chromosomes produced by the passage of the spindle fiber attachment regions to opposite poles introduces a strain on the chromosomes which eventually leads to breakage. In the case of the double-sized ring with the two spindle fiber attachment regions, the chromosome does not always break in the middle but may break in several places, the parts adjacent to the spindle fiber attachment region at both poles passing into the telophase nuclei, the other parts being left in the cytoplasm. Obviously, then, the chromatin content and structural arrangement of the original ring has become altered. It might be expected that this mechanism would produce rod-shaped chromosomes. On the contrary, the broken ends thus produced apparently reunite to form rings."

The assumption of the occurrence of a chromosome split in more than one plane is necessary to explain the behavior of persisting ring-shaped chromosomes that are continually breaking if the chromonema is single at mitotic anaphase, and the "permanent" union of broken ends is accomplished before any relational coiling of chromatids can occur. In Tradescantia, and in other plants with large chromosomes, there can be no doubt that the mitotic telophase chromosomes are at least two-parted. There is considerable evidence that they are four-parted (NEBEL 1932; STEBBINS 1935; GOODSPEED, UBER and AVERY 1935). The anaphase chromosomes of the second meiotic division in Tradescantia are two-parted (NEBEL 1932; KUWADA and NAKAMURA 1935; SAX and SAX 1935; DERMEN 1936). All the ring-shaped chromosomes which were found at the first division of the microspore in Tradescantia were formed, therefore, after the chromosome split had occurred. One or both daughter chromosomes resulting from the majority of these rings will break at anaphase. At the time the breakage occurs, the daughter chromosomes will be at least two-parted, as was the parent ring at the time of its formation. A broken end of an anaphase chromatid may unite as often with the broken end of its sister as with its other broken end. Ring chromosomes which are continually breaking might persist in this way. Whenever the two broken ends of one chromatid unite, however, and the two sister strands are *not twisted*, a "disjunctional" ring-shaped chromosome would result. There would be a trend toward replacement of the "continuous" and "interlocked" types by "disjunctional" rings which separate without breaking unless relational coiling of chromatids is increased before each union of broken ends. If the complete union of broken ends is delayed until somatic prophase, until after a relational coiling of chromatids has taken place, the behavior of ring-shaped chromosomes which are persistently breaking becomes understandable without assuming that the chromosome split occurs in more than one plane. The chromatids of these chromosomes, especially the larger ones, may become relationally coiled and the broken ends fused completely at prophase after each breakage that is brought about during the previous anaphase separation. Chromosomes with two attachments should behave in the same manner.

SUMMARY

During the mitotic cycle the two chromatids of each chromosome are twisted about each other or relationally coiled. The amount of relational coiling during the preceding anaphase and late prophase and metaphase in microspore development of Tradescantia has been studied directly. During the resting stage, and early prophase, direct analysis is impossible, so these stages have been studied by means of X-ray induced chromosome configurations which retain their relational coiling until they break at anaphase.

Among the abnormalities obtained after X-ray treatment were chromosomes with two fiber attachments and ring-shaped chromosomes. The amount of relational coiling was approximately the same in both of the configurations. A study of these chromosomes shows that the reduction of relational coiling in a normal chromosome does not involve the rotation of chromatids at the fiber attachment point. The proportions of free and locked associations differ in the two types and can be attributed to the fact that the direction of relational coiling is at random for any two chromosomes, but tends to be in the same direction in the two arms of a single chromosome which fuse to form rings.

The amount of relational coiling at metaphase in the ring-shaped chromosomes appears to be the same, regardless of the time of X-ray treatment between telophase and early prophase. This evidence suggests that the union of broken ends of chromosomes is not effected at the time of X-ray treatment, but occurs at a certain stage in prophase development. Such behavior would explain the persistence of ring-shaped chromosomes which are continually breaking.

The available evidence indicates that there is little relational coiling of chromatids at anaphase during mitosis, an increase during prophase as new minor spirals are formed, and a decrease during very late prophase and metaphase as each chromatid becomes shortened, more rigid, and the formation of minor spirals is completed. In the meiotic prophase relational coiling is not initiated until homologous chromosomes are paired.

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THE RELATIONS OF INVERSIONS IN THE X CHROMOSOME OF DROSOPHILA MELANOGASTER TO CROSSING OVER AND DISJUNCTION*

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INTRODUCTION

STUDIES of chromosome aberrations such as polyploidy and translocations have contributed much to the understanding of the meiotic behavior of chromosomes. One of the commonest types of structural difference in chromosomes within a species is that in which a segment of a chromosome has been inverted. These cases have not contributed as much as might have been expected toward an understanding of chromosome mechanics. It has been apparent for some time that they needed systematic study, and that the series of X chromosome inversions accumulated in X-ray experiments furnished the necessary material. The present paper represents the results of a study made with these points in mind.

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Recent papers on inversions illustrate the difficulties encountered in such studies. Gershenson (1935) and Stone and Thomas (1935) found that the chromatids resulting from single crossing over are not recovered, and by making egg counts showed that there is no detectable mortality that can be invoked to account for them. They concluded that single exchange is so rare as to be negligible. Sidorov, Sokolov, and Trofimov (1935) showed by the use of attached-X females that single exchanges do occur with a high frequency; but they appear to have been unaware that egg counts fail to show any corresponding mortality. The dates show that at least the second and third papers mentioned and our own preliminary note (Beadle and Sturtevant, 1935) were all sent to press independently. Our account, of which the present paper is the full presentation, included both the proof that single exchanges occur (using the same method as the Russian investigators), and the proof that there is no corresponding egg mortality. We were faced with a seeming paradox, the only escape from which was the assumption that the single crossover chromatids are produced but are not included in the egg nucleus. We developed a scheme that gives this result (pp. 591–596, and figs. 6 and 7), and that is in good quantitative agreement with the data in other respects.

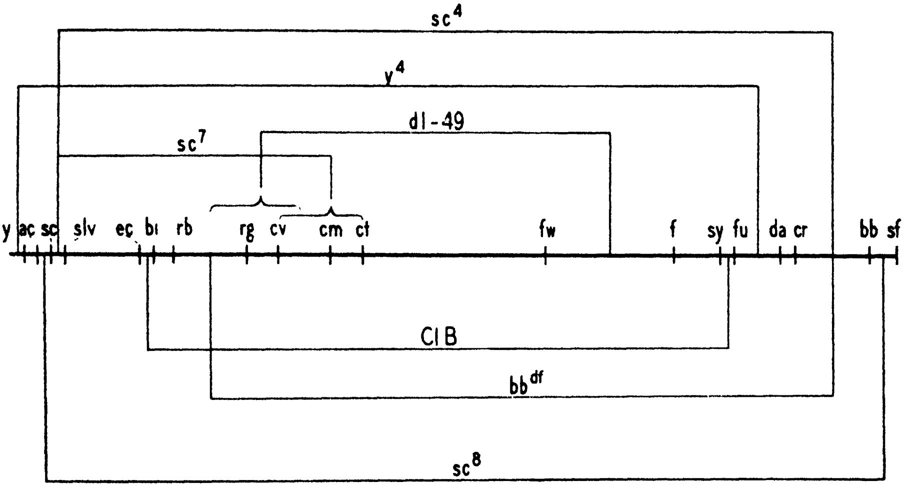


Figure 1 — Diagram showing the nature of the inversions used in the present study.

INVERSIONS STUDIED

There follows a descriptive catalogue of the inversions we have studied. Figure 1 shows the lengths of these inversions. The data on the extent of each inversion are largely from the experiments with females heterozygous for two inversions, which are described below. The egg mortalities associated with the inversions are given in a later section.

Inversion scute-4

Agol (1929) subjected yellow flies to X-rays, and obtained an extreme scute allelomorph, sc^4. This was found to be associated with a long inversion in the X chromosome, shown below to extend from a point between *sc* and *slv* to a point between *cr* and *bb*. Females heterozygous for this inversion give about 9 percent crossing over among their regular offspring, and also produce about 6 percent of patroclinous sons, as shown in table 1. All the recovered crossovers are doubles.

Our best experiments are those in which *y*, *cv*, *v*, and *f* are followed, since here it is probable that few or no undetected doubles occur. The table shows a few crossovers entered as singles; these are evidently all really doubles in which the second crossover has occurred in the unmarked region between *f* and the inversion point. In another experiment, in which *cr* and *bb* were present, but the rest of the chromosome was not so well controlled, two such apparent singles were tested and found in fact to represent *cr bb* crossovers as well.

Table 1 shows a total of 108 crossover males to 63 patroclinous from XX females; the ratio between these classes, 1.71:1, will be discussed below.

Table 1

Tests of females heterozygous for inversion sc-4. In this and following tables the crossover classes are labelled according to the standard sequence of loci, unless otherwise indicated In cases where two contrary classes are entered under one heading, the right-hand or lower one of the two carries the mutant gene at the leftmost locus concerned.

MOTHERS	♀		REGULAR ♂♂																EXC. ♂♂
	REG.	EXC.	0		1		2		3		1, 2		1, 3		2, 3		TOTAL	
$sc^3\ cv\ v\ f/y\ sc^4$	344	0	115	150	1	2	2	1	1	0	1	0	3	5	4	6	291	18
$y^2\ cv\ v\ f/y\ sc^4$	331	0	154	86	3	0	0	0	1	2	1	0	4	7	5	3	266	14
$v/y\ sc^4\ cv\ f$	801	1	294	291	2	3	7	6	0	2	3	2	10	11	6	4	641	31
$y^2\ cv\ v\ f/y\ sc^4/Y$	397	11	144	105	3	1	4	3	1	4	3	2	9	9	4	7	299	52

The total crossover percentage among regular sons is 9.3 from the XX females, 16.7 from the XXY females. The latter value needs correction, however. Half of the exceptional gametes die, so the totals should be taken as regulars plus twice the exceptional females; this gives $50:299+22$, or 15.6 percent. It seems clear that the presence of a Y significantly increases the frequency of recovered double crossovers.

Crossing over in sc-4/sc-4 is approximately normal. Three experiments are recorded (table 2). The third experiment also gave a total of 14 reversions of Bar and 6 occurrences of double-bar (in 8,523 flies), all of them *f-fu* crossovers. In many of these cultures the *B* flies were not classified for *f-fu*. This serves to confirm the report of Muller and Weinstein (1933), based on sc-8 experiments, that unequal crossing over occurs only between non-sister strands even when the *B* locus is far removed from the spindle attachment.

Table 2

Crossing over in homozygous inversion sc-4. Regular males only are recorded in the first experiment; in the second and third both males and females are included.

MOTHERS	0	1	2	3	1, 2	1, 3	2, 3	TOTAL
cv cr/w^e f	66 73	14 8	43 42	9 16	2 3	1 1	1 2	281
v f/cv	93 94	31 20	24 37		3 0			302
B/f B fu	2707	103						2810

Inversion scute-7

Dubinin (1930) has described scute-7. It was obtained by X-raying apricot (w^a) flies, and is a recessive scute allelomorph resembling but definitely different from scute-1.

Females heterozygous for scute-7 have shown no crossing over for any loci to the left of *ct*, and a reduction of crossing over for the interval from *ct* to *lz*. To the right of *lz* substantially normal values have been obtained (table 3). Tests of crossovers have shown that the decrease of crossing over is due to something lying to the left of *sn*, in the region most affected.

Table 3*

LOCI	sc-7/+		sc-7/sc-7	
	N	PERCENT	N	PERCENT
sc-w	6213	0	—	—
w-cv	3103	0	—	—
fa-sn	—	(0.4)	603	2.8
cv-ct	1030	0	—	—
ct-sn	1395	0.4	—	—
cv-v	938	7.5	—	—
sn-lz	1395	1.6	292	5.1
lz-v	—	(5.5)	292	3.4
v-f	678	24.9	350	22.6

* These values are all from XX females. Less extensive data are available for XXY females in the case of sc-7/+; they show no significant differences from the above values.

Owing to the complete absence of crossing over to the left of *ct* it was at first impossible to test this region in homozygous sc-7. However, in a sc-7 chromosome a mutation occurred to an allelomorph of facet, found in an X-ray experiment by Mrs. C. E. Ruch. The results obtained with this chromosome, tested against genes introduced by crossing over (table 3), show that the reduction of crossing over in heterozygous sc-7 is due to an inversion that includes *fa* but not *sn*.

The extent of this inversion has not been determined accurately by genetic methods, since it does not give viable crossovers with any other inversion we have used, unless special methods are used. Salivary gland chromosomes have, however, been seen to give typical heterozygous inversion figures that show the existence of a short terminal uninverted piece. Dr. J. SCHULTZ has studied such preparations more carefully, and we are indebted to him for the information that the left inversion point lies close to the right of scute, the right one not far from the locus of crossveinless.

Inversion scute-8

SIDOROV (1930) subjected apricot (w^a) flies to X-rays and obtained a bristle mutant described as a new scute allelomorph. The relations of this to the scute and achaete series are peculiar; but it is most convenient to retain the name scute-8 for it. There is an associated inversion which has been studied by several investigators (PATTERSON and STONE 1935; STONE and THOMAS 1935). These authors have described its properties; the data given here are in essential agreement with their account.

As will be shown below, the inversion extends from a point between *ac* and *sc* to a point to the right of *bb*. Its right end has been shown by GERSHENSON to be located in the inert region of the X.

The crossing over shown by sc-8/+ is illustrated in table 4. Our other experiments are less satisfactory in that fewer or less well-spaced loci are concerned, but they agree in indicating that these are about the usual values. As in other cases, the classes listed as single crossovers are obviously really doubles, with the second crossover between forked and the right inversion point.

No adequate data are available for crossing over in XXY sc-8/+ females.

Table 4 shows that crossing over is of about the normal frequency in sc-8/sc-8, though (as briefly stated by OFFERMAN and MULLER, 1932) there are local differences from the standard values. The w^a-*cv* interval gives 6.1 percent, as opposed to the usual value of about 12 percent, whereas *f-cr* gives 9.5 as opposed to about 6 percent. Thus in both cases the same section gives more crossing over when it lies far from the spindle attachment.

Inversion scute-8 deficiency

The scute-8 inversion reaches from a point between *ac* and *sc* to a point to the right of *bb*. We have found, as NOUJDIN (1935) has recently reported, that any series of sc-8, in which appropriate tests are made, produces occasional *y-ac* deficiencies, that is the left inversion point involves an unstable union of parts. The resulting chromosome is deficient for *y*, *ac*, and probably *Hw*, and for no other known loci. All the remaining known loci are in reverse sequence. We have used this "Df sc-8" in some of our inversion experiments.

TABLE 4

Crossing over in inversion sc-8 females.

	In/+		In/In			
CLASSES	sc-8 w^a cv v f / y^2	ct / sc-8 w^a cr	w^a ct cr / cv f	+ / w^a cv v f	w^a cv / v f	w^a v / cv f
0	764 913	341 277	490 509	407 257	173 123	78 79
1	0 0	1 1	33 47	26 31	13 11	9 5
2	4 3	1 4	29 42	97 75	52 27	18 21
3	9 14		345 326	110 94	54 43	25 20
4	0 4		74 71			
1, 2	0 0	6 14	0 1	1 1	0 1	0 0
1, 3	0 0		13 13	3 5	4 2	4 4
1, 4	0 1		6 9			
2, 3	2 3		9 10	18 5	10 2	3 1
2, 4	8 12		6 2			
3, 4	20 13		12 15			
1, 3, 4	0 0		0 1			
2, 3, 4	0 0		0 1			
Exc. males	57	21				

As in the cases of sc-4/+ and sc-8/+, the apparent single crossovers are evidently all really doubles, in which the second crossover was to the right of *f*. The relative numbers of double crossover males (30) and patroclinous males (31) need correction for comparison with other series, since only half the crossovers survive while the other half carry the deficiency. As thus corrected the ratio becomes 60:31 = 1.9:1.

Tables 4 and 5 indicate that there are about twice as many crossovers recovered from Df (sc-8)/+ as from sc-8/+. Analysis of a few other crosses, not here reported in detail because they include fewer genes or for similar reasons, suggests that the difference, if present, may be less than indicated. More experiments are needed.

TABLE 5*

$$\frac{sc\ cv\ v\ f}{\text{Df (sc-8) } w^a} \times y^2\ cv\ v\ f$$

INTERVAL	MALES	FEMALES	
0	211	245	245
1	2	4	5
2	2		
3	7	16	9
4	1	1	6
1, 3	0	1	1
2, 3	0		
1, 4	1	2	5
2, 4	7		
3, 4	10	8	7
Total	241	555	
Ex.	31		

* Df sc-8 is lethal, so that each crossover class of males is represented only once. The females could not be classified for w^a, so intervals 1 and 2 were not separable in them. In the female row the not-yellow class is entered to the left in each case. Egg counts made by Miss M. Groscurth show that the males carrying Df sc-8 die in the egg stage.

Females of the constitution Df (sc-8)/sc-8 have all their genes in the same sequence, but are heterozygous for a terminal *y-ac* deficiency. Table 6 gives the crossing over observed.

TABLE 6

Crossing over in sc^8B/Df (sc-8) *cv v f* ♀♀.*

0		1		2		3		4		1.2		1.3		2.3		TOTAL
89	62	26	16	8	6	2	5	0	0	29	2	25	27	7	9	332

* This table gives the results of a cross to *y cv v f*; it includes both sexes, and contrary classes are therefore not equivalent because of the lethal nature of the deficiency. In reality the sequence is Df *B f v cv*; the classes above, renumbered on this basis, become, in the order given: 0; 1; 1.4; 1.3; 1.2; 4; 3; 3.4).

From table 6 values for comparison with sc-8/sc-8, have been calculated:

	w^a-*cv*	*cv-ct*	*cv-v*	*v-f*	*f-B*	*f-cr*	*B*-Df
sc-8/sc-8	6.1	4.8	17.4	21.3	—	9.5	—
sc-8/Df (sc-8)	—	—	24.1	22.6	0	—	19.0

In the case of *cv v* the data suggest a difference; other experiments (involving fewer loci) with sc-8/Df (sc-8) give values of 18.7 (in 215 flies) and 16.6 (in 380 flies). Both series are therefore to be taken as giving not far from 18 percent.

Inversion ClB

The "ClB" chromosome was first briefly described by Muller (1928), and has been very extensively used since in special experiments. The most detailed account of its properties is that given by Gershenson (1935). Painter (1934) has figured the inversion that is visible in the salivary-gland cells of ClB heterozygotes. As described by Muller, this chromosome has an inversion (shown below to extend from a point between *ec* and *bi* to a point between *sy* and *fu*), and carries a lethal and the mutant genes *sc*, *t*, *v*, *se*, and *B*. The leftmost of these, *sc*, lies outside the inversion and separates from it by crossing over occasionally. The other mutant genes lying in the inversion are also occasionally lost by double crossing over. We should interpret the "reinverted" ClB chromosome described by Gershenson (1930) as having arisen from a triple crossover—a double within the inversion and a simultaneous single to the left of it. Gershenson (1935) records a total of 1 percent crossing over for the whole chromosome (*sc* to *cr*), a value that is, if anything, slightly higher than general experience would lead one to expect. About three-fourths of this crossing over is made up of singles to the left of the inversion. Our own data are less extensive than Gershenson's, and need not be presented since they add nothing of importance.

Gershenson has also studied crossing over in XXY females heterozygous for ClB. He found, as have we, that the exceptional females produced are practically always non-crossovers. After correcting his observed crossover values by adding the non-disjunctional eggs to the observed non-crossover regulars, he arrived at the conclusion that approximately the same amount of crossing over occurs as in XX females. His values are 1.29 percent for crossing over to the left of the inversion, .07 percent for doubles within the inversion, the differences from the XX values being opposite in sign and insignificant in amount; the percentages are, however, too small to be useful for such comparisons.

Gershenson has added one other essential bit of evidence, concerning a relation we have not studied. He determined (the method used is not stated) the frequency of XX and XXY females among the regular daughters of XXY ClB females. His table IV shows that among the regulars carrying the ClB chromosome there were 47 XXY:49 XX; among those carrying the non-inverted chromosome there were 68 XXY:63 XX. Evidently, in the regular eggs the Y is distributed at random.

A similar relation was recorded by BRIDGES (1916) for XXY females carrying no inversion, half the regular daughters being shown to be XXY, the other half XX. KIKKAWA (1932) reports that in *D. virilis*, significantly less than half are XXY. This result is based on cytological observations and seems to us to be doubtful. The Y of *virilis* is not visibly different from the X's or from eight of the autosomes; under these circumstances wholesale counts made on the minute oögonial chromosomes seem questionable.

TABLE 7*

$$♀\ \frac{\text{dl-49}\ cm^2\quad bb}{v\ cr} \times ♂\ v\ B\ bb$$

	XX ♀♀		XXY ♀♀	
Regular (*B*) ♀				
0	220	201	134	255
1	30	40	152	57
Exc. (+) ♀	0		578	
Reg. (not-*B*) ♂				
cm or *cr* (*v* or not-*v*)	419		567	
v	19		40	
Exc. (*v B*) ♂	1		480	

* In the male classes *bb* cannot be identified, and *cm* and *cr* are not separable. The *v-cr* crossing over can only be determined by doubling the *v* class of males, the contrary crossover, *cm cr*, not being identifiable. In the female classes the crossing over detected is that between *v* and *bb*; in the case of the XXY females *bb* is suppressed by the Y present in some of the regular daughters so the observed classes are misleading. Using *bb* classes only the numbers are: non-crossovers 134, crossovers 57.

Inversion delta-49

Inversion dl-49 was first described in an abstract by MULLER and STONE (1931). The left break was shown to lie between *rb* and *cm*, the right between *fw* and *f*. According to PAINTER (1934) unpublished data of these workers show that the right break lies between *fw* and *g*. PAINTER (1934) showed from a study of salivary chromosomes of females heterozygous for inversion dl-49 that the left break occurred between *rb* and *cv*. Since the method that we have used for determining the ends of inversions could not (with the material at our disposal) be used with dl-49, we can add no further information.

Females heterozygous for Inversion dl-49 give few or no crossovers within the inversion among their offspring. We have not collected any extensive series of data showing this to be true since it seemed quite unnecessary. Inversion dl-49 is extensively used in "balanced" stocks and, so far as we know, no case of double crossing over within the inverted segment has occurred in heterozygous females; the opportunities for detecting such, had they occurred, have been abundant.

Females homozygous for inversion dl-49 show approximately normal crossing over both within the inversion and outside it, according to STONE and THOMAS (1935).

Females heterozygous for inversion dl-49 do give recoverable crossovers outside the limits of the inversion, those to the left occurring with considerably lower frequency than those to the right. Among 533 progeny of the cross inversion dl-49 $cm^2\ f/y\ ec\ cv\ ct^6\ v\ g^2\ f \times y\ ec\ cv\ ct^6\ v\ g^2\ f$, 3 or 0.56 percent were crossovers between *y* and *ec*.

The data of table 7 show, from XX females, *v-cr* = 8.7 percent, *v-bb* = 14.3 percent. The latter value may be taken as giving the amount of crossing over between the inversion and the spindle attachment; it is in reasonable agreement with the value of 13.4 obtained by SCHULTZ (quoted by L. V. MORGAN 1933).

Table 7 also shows the crossing over from XXY females heterozygous for inversion dl-49. After correcting for the inviable non-disjunctional gametes the values are *v cr* = 4.5, *v bb* = 10.1. Another series of XXY females, of the constitution dl-49 $cm^2\ bb/y^2$, gave (corrected) $y^2cm^2 = 0.5$, $y^2bb = 13.4$, based on 275 regular males and 70 *bb* regular females, respectively. The conclusion seems warranted that the Y somewhat decreases crossing over between the spindle attachment and the inversion.

Inversion yellow-4

According to DUBININ and FRIESEN (1932), the y-4 inversion was found by SEREBROVSKY. It presumably arose as the result of X-ray treatment and is inseparably associated with a mutation of the yellow gene to an allelomorph very closely resembling the original y^1. As shown below, the leftmost inversion break is located very near to or at the yellow locus (to the left of it according to MULLER and PROKOFJEVA 1935); the rightmost break is located between the genes *fu* and *da*.

Females heterozygous for inversion y-4 give, among their regular offspring, about 2.7 percent of double crossovers within the inversion; about 2.4 percent of the sons of such females are patroclinous. The data from which these percentages are obtained are presented in table 8. Certain of the crossovers appear as singles within the inversion; they are presumably doubles with the second crossover in the short uncontrolled region between *f* and the end of the inversion. The ratio of recovered double crossovers among male offspring of XX mothers (57) to patroclinous males (51) is 1.12:1.

The data from XXY females (table 8) can be compared with those from XX females, since the XXY mothers were sisters of approximately half of the XX mothers and since the two lots of XX data gave similar results (2.8 and 2.3 percent of males patroclinous, 2.7 and 2.0 percent of regular males crossovers). The percentage of crossovers among regular sons of XXY mothers is 6.1, or 5.8 when corrected for exceptional offspring. As in the case of inversion sc-4, the frequency of recovered double crossovers is increased by the presence of a Y chromosome.

TABLE 8

Crossing over in In y-4/sc cv v f and in sister In y-4/sc cv v f/Y females by B or sc B males.

	XX FEMALES		XXY FEMALES	
B ♀♀	2106		548	
+ ♀♀	1		39	
B ♂♂	51		56	
Regular males				
0	1019	1044	287	250
1–2	2	3	3	3
1–3	16	11	9	9
1-(4)	0	1	1	0
2-3	11	8	7	6
2-(4)	3	1	0	0
3-(4)	0	1	0	0
Total regular males	2120		575	

Inversion bobbed deficiency

From X-ray treated males Sivertzev-Dobzhansky and Dobzhansky (1933) got an X chromosome carrying a deficiency for the proximal third of the somatic metaphase X chromosome, and a small-wing (*sl*) allelomorph. Females heterozygous for this and a normal X chromosome gave very low crossover values, a result ascribed by the above workers to the presence of the deficiency in heterozygous condition. We have obtained clear evidence that the bobbed deficiency chromosome carries an inverted segment extending from between *rb* and *rg* to between *cr* and the spindle attachment.

Sivertzev-Dobzhansky and Dobzhansky published data on crossing over in females heterozygous for Df (bb). Our data are substantially the same (table 9). The *y-ec* interval lies outside the inversion and shows (in XX females), 0.8 percent of recovered single crossovers. This is a marked reduction as compared with the standard crossover value for this interval. The region from *ec* to *ct* is partly outside and partly within the inversion. To get the frequency of recovered double crossovers within the inversion, apparent singles in this region (four in number) are assumed to be actually doubles since the greater part of the region must lie inside the inversion. Recovered doubles within the inversion, assuming apparent singles to be doubles with the second single in the unmarked *f*-spindle attachment region, constitute 3.9 percent of the regular males. The relation between double crossovers and patroclinous males will be considered in connection with the mechanism of disjunction in inversions.

TABLE 9

Data from the cross $sc\ sl^2\ Df(bb)/ec\ ct^6\ g^2\ f \times w\ B$. *The XX and XXY females were sisters*

	XX FEMALES		XXY FEMALES	
B ♀ ♀	1244		1622	
+ ♀ ♀	0		255	
wB ♂ ♂	21		292	
Regular males				
0	443	601	695	774
1	5	4	4	10
2-(6)	1	3	5	2
3-(6)	12	10	13	17
4-(6)	1	2	4	1
5-(6)	1	0	2	0
2-3	0	0	1	0
2-4	0	0	0	1
2-5	0	1	2	1
3-4	1	6	3	0
3-5	3	1	5	3
4-5	1	0	0	0
1-3-(6)	0	0	0	
Total regular males		1096		1544

Females heterozygous for Df (bb) and carrying a Y chromosome gave about the same frequency of double crossovers within the inversion as did their XX sisters, 3.7 (corrected for non-disjunction) as compared with 3.9 percent. There is no indication here of an increase in crossing over in the presence of a Y chromosome such as that shown by the sc-4 and y-4 inversions.

Crossover data from XXY females homozygous for Df (bb) are given in table 10. (XX homozygotes do not survive, as shown by SIVERTZEV-DOBZHANSKY and DOBZHANSKY.) The *y-f* interval gives a crossover value of 37.1 percent which is higher than is given by this segment in its normal position in the chromosome (8 plus 10 units). The remaining intervals show less than normal crossing over with the decrease becoming more marked toward the spindle attachment. Presumably we are here dealing with the so-called spindle attachment effect, that is, segments moved away from the spindle attachment show increased crossing over; distal segments moved near the spindle fiber show a decrease in crossing over (OFFERMAN and MULLER 1932; BEADLE 1932).

TABLE 10

Data from the cross $y^2\ f\ v\ Df(bb)/sc\ g^2\ ct^6\ Df(bb)/Y \times w\ B$.

		B ♀♀	2269		
		+ ♀♀	69		
		w B ♂♂	84		
Regular males					
0	478	434	1–4	15	17
1	304	312	2–3	0	2
2	72	107	2–4	3	5
3	46	52	3–4	1	1
4	33	37	1–2–4	1	1
1–2	23	19	1–2–3	1	0
1–3	25	27			
			Total regular males		2016
		Region	Percentage of crossovers		
		1	37.1		
		2	11.6		
		3	7 7		
		4	5.7		

COMBINATIONS OF DIFFERENT INVERSIONS

In females carrying overlapping inversions, single crossovers within the region common to the two inverted segments should give chromatids with single spindle attachments, in contrast to the chromatids with two or with no spindle attachments resulting from single crossing over within the inverted segment in a female heterozygous for a single inversion. If two overlapping inversions are not too different in length such single crossovers should be viable in the heterozygote. Actually we know this to be the case in several combinations of X chromosome inversions. GERSHENSON (1932) has reported a bobbed deficiency chromosome resulting from single crossing over between In sc-4 and In sc-8.

Crossovers between different inversions will of course give different results depending on the relative positions of the inversion points. Thus, representing the normal sequence of segments of a chromosome such as $A\ B\ C\ D\ E\ F$, inversions differing only at one end, $\dfrac{A\ B\ D\ C\ E\ F}{A\ B\ E\ D\ C\ F}$, will give crossovers $A\ B\ D\ C\ F$, a single deficiency and $A\ B\ E\ D\ C\ E\ F$, a single net duplication (fig. 2). If both ends differ in position, there are two more possibilities. Thus $\dfrac{A\ B\ D\ C\ E\ F}{A\ E\ D\ C\ B\ F}$ gives $A\ B\ D\ C\ B\ F$, a duplication for *B* and a deficiency for *E*, and the complementary duplication-deficiency *A E D E F* (fig. 3). The third possibility $\frac{A\ B\ E\ D\ C\ F}{A\ D\ C\ B\ E\ F}$ gives the double duplication *A B B D C B E F* and the double deficiency *A D C F* (fig. 4). We shall consider examples of all of these possibilities.

TABLE II

Chromosomes resulting from single crossing over within common inverted regions.

SOURCE OF				♀	♂
LEFT END	RIGHT END	DUPLICATION FOR	DEFICIENCY FOR	HETEROZYGOUS FOR NORMAL CHROMOSOME	
sc-4	sc-7	*ct-cr*	none	Inviable	Inviable
sc-4	sc-8	*sc*	*bb*	Normal; fertile	Normal; fertile; inviable without Y
sc-4	Clb	*fu-cr*	*slv ec*	Inviable	Inviable
sc-4	dl-49	*g-cr*	*slv-rb*	Inviable	Inviable
sc-4	y-4	*y-sc, cr*	none	Normal	Normal
sc-7	sc-4	none	*ct-cr*	Inviable	Inviable
sc-7	sc-8	*sc*	*ct-bb*	Inviable	Inviable
sc-8	sc 4	*bb*	*sc*	Fertile; legs often abnormal	Nearly completely lethal, sterile; extreme sc
sc 8	sc 7	*ct-bb*	*sc*	Inviable	Inviable
sc-8	Clb	*fu-bb*	*sc-ec*	Inviable	Inviable
sc-8	dl-49	*g-bb*	*sc rb*	Inviable	Inviable
sc-8	y-4	*y-ac,* *cr-bb*	none	*Hw* effect of sc-8, fertile	*Hw* effect of sc-8
Clb	sc 4	*slv ec*	*fu-cr*	Inviable	Inviable
Clb	sc-8	*sc-ec*	*fu-bb*	Inviable	Inviable
Clb	dl-49	*g-sy*	*bi-rb*	Inviable	Inviable
Clb	y-4	*y-ec*	*fu*	Fertile; abnormal eyes, wings, hairs	Inviable
Clb	Df(bb)	none	*bi-rb,* *fu-bb*	Poorly viable; sterile; minute bristles	Inviable
dl-49	sc-4	*slv-rb*	*g-cr*	Inviable	Inviable
dl-49	sc-8	*sc-rb*	*g-bb*	Inviable	Inviable
dl-49	Clb	*bi-rb*	*g-sy*	Inviable	Inviable
dl-49	y-4	*y-rb*	*g-fu*	Inviable	Inviable
y-4	sc-4	none	*y-sc, cr*	Poorly viable; fertile; minute bristles	Inviable
y-4	sc-8	none	*y-ac* *cr-bb*	Poorly viable; fertile; minute bristles	Inviable
y-4	Clb	*fu*	*y-ec*	Inviable	Inviable
y-4	dl-49	*g-fu*	*y-rb*	Inviable	Inviable
y-4	Df(bb)	none	*y-rb cr*	Inviable	Inviable
Df(bb) .	Clb	*bi-rb,* *fu-cr*	none	Fertile; wings slightly narrowed	Sterile; wings notched at tip
Df(bb)	y-4	*y-rb, cr*	none	Fertile; wings narrow, bristles abnormal	Inviable

The recoverable single crossovers with either one or two deficient segments are useful in determining genetically the position of the inversion points. Thus if such a crossover shows a deficiency for gene B but not for genes *A* and *C*, we can say that one inversion end is located between genes *A* and *B*, the other between *B* and *C*. The precision of this method is limited only by the extent and accuracy of the genetic map and by the fact that only recessive mutant effects are available for deficiency tests.

From many combinations of two inversions we have collected data on non-disjunction (table 14) which are discussed below.

Table 11 gives a summary of the available information concerning the properties of the chromosomes derived from single crossing over in the region common to two inversions. Where we have recorded a given crossover chromosome as being inviable, this is to be understood as meaning "under ordinary conditions." It is quite likely that some of the types in question could be brought to maturity by special culture techniques, which we have not used in any case.

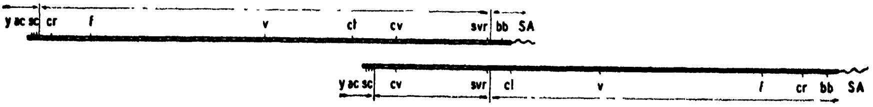


FIGURE 2.—Inv sc-4 (above)/Inv sc-7 (below). Diagram showing conjugation of the common inverted regions. The arrows point in the direction in which the loci are arranged in "normal" chromosomes, reading from the distal end to the spindle attachment.

Inversion sc-4/Inversion sc-7

In this case both left inversion points lie very close to *sc*, and to the right of it; they must be nearly or exactly at the same level. The right point is much further from this level in sc 4. Singles within the common inverted region would be either duplications or deficiencies for the long section from near *cv* to *cr* (fig. 2), and it is accordingly in agreement with expectation that they were not recovered in crosses to normal males. The only other type of crossover that might be expected to appear is the double within this *cv-cr* section, and a few of these were obtained.

From $y\ sc^4\ cv\ f/sc^7\ w^a$ ♀ (×various ♂♂) were obtained 459 regular ♀♀ (no crossovers were observed in those 2 of the 4 cultures where they could have been identified, but detailed counts were not recorded), 228 $sc\ w^a$ ♂♂, 176 *y sc cv f* ♂♂, 8 $sc\ w^a\ f$ ♂♂, 3 *y sc cv* ♂♂,

By mating sc-4/sc-7 heterozygotes to translocation 1,2-7 (break between *rb* and *cv* in X and attached to the right of *sp* in II) or translocation 1, 3-3 (break between *rb* and *cv* in X, attached to the right of *ca* in III) we were able to save the crossovers in the common inverted segment which are deficient for the long segment from the right end of the sc-7 inversion (near *cv*) to the spindle attachment (fig. 2). From the cross *y* sc^4 *v f cr*/ sc^7 w^a fa^2 *sn v* by T 1, 2–7, 455 normal ♀♀, 2+♂♂, 181 *y* sc^4 *v f cr* ♂♂, 184 *sc* w^a *fa sn v* ♂♂ were obtained. In addition there were 16 males carrying the deficiency crossover plus the proximal X segment from the translocation. Of these 13 were *sc*, 1 was *sc* w^a *fa* and 2 were *sc fa*. All were strong scutes, otherwise normal. Of these 11 were tested; none was fertile (they are of course expected to be XO). The 16 males constitute 4.05 percent of the regular males, which would indicate, since the contrary class is lost, a frequency of 8.1 percent crossing over in the common inverted segment. It is assumed that the translocation males produce four types of gametes in equal numbers.

Females of the same constitution as above mated to T 1, 3–3 males gave essentially similar results. There were 407 regular non-crossover males and 6 crossovers of the type considered above. Here the frequency of single crossovers, corrected for the class not recovered, is 2.9 percent. Three of these males were tested and, as expected, were sterile.

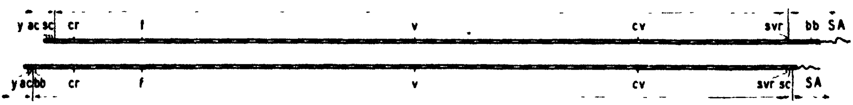


FIGURE 3.—Inv sc-4 (above)/Inv sc-8 (below). Conjugation of the common inverted regions.

In both of the above cases the percentages of recovered crossovers indicate minimum values of crossing over in the common inverted segment. Since the recovered crossover individuals carry a net duplication (difference between sc-7 right break and translocation break) they are probably lower in viability than non-crossover males. Likewise if the translocations give more regular than non-disjunctional gametes the observed frequency of crossing over will be lower than the real value.

Inversion sc-4/Inversion sc-8

The sc-8 inversion is slightly longer than the sc-4 one at both ends, the resulting single crossovers carrying either a deficiency for *sc* and a duplication for *bb* or a duplication for *sc* and a deficiency for *bb*. The conclusions as to their limits depend in part on the results obtained from the heterozygote here under discussion, so the argument may now be presented.

GERSHENSON (1932) has already described the crossover that receives the left end of sc-4 and the right end of sc-8 (fig. 3), showing that it acts as though it carried a lethal allelomorph of *bb*, both the lethal and the bobbed effects being suppressed by a Y. This shows that the *bb* locus is absent, that is, that it is present in the inverted portion of sc-8 and in the uninverted portion of sc-4, both of which are absent in this crossover.

GERSHENSON has also studied the deficient chromosome cytologically in the oögonia of heterozygous females; he finds it to be reduced in length by about one quarter. This can only mean that a large section (not far from half) of the inert region is included in In sc-8 and not in In sc-4. The latter presumably includes little or no inert region. Finally, GERSHENSON showed that this chromosome did not carry a deficiency for *cr*. It follows that In sc-4 has its right break between *cr* and *bb*, while In sc-8 has it to the right of *bb*, conclusions which our own data confirm.

The other crossover, that must carry two *bb* loci, is usually lethal in the male. We have found that it does not carry a deficiency for *ac*, *slv*, or *br*. These three loci must thence be alike in the two inversions, that is, either in both or outside of both, since the crossover studied by GERSHENSON also carries no deficiency for them. When tested against scute allelomorphs this chromosome behaves as an extreme scute, but so does sc-4 itself, so this is not a critical result. However, we have been able to obtain a few males carrying this chromosome. Occasionally they emerge but only live a few hours. Examination of their bristles shows that they have many fewer than sc-4 males; the only named ones observed were the inner verticals, posterior supra-alars, and the dorsocentrals, that is, the "achaete" as opposed to the "scute" bristles, and even these were frequently absent. Dr. J. SCHULTZ informs us that a study of the salivary gland chromosomes also indicates that the sc-8 break is to the left of the *sc* band, the sc-4 one to the right of it. It must be concluded that these males represent the occasional survival of specimens in which the scute locus is wholly absent.

Females heterozygous for this chromosome often have some of their legs abnormal. The abnormality, which is most frequent in the posterior pair, may consist in bifurcation, shortening and twisting, or basal fusion of the two members of one pair.

The above results show that In sc-4 runs from a point between *sc* and *slv* to a point between *cr* and *bb*; In sc-8 from a point between *ac* and *sc* to a point between *bb* and the spindle attachment.

TABLE 12

	$\frac{f\ v\ cv\ w^a\ (\text{sc-8})}{y\ sc^4\ \cdot}$ × various ♂♂				
♀					
Except. ♂♂	456		1, 2	1	3
Regular ♂♂	0		1, 3	7	3
0	52	76	1, 4	1	1
1	0	28	2, 4	2	3
2	1	37	3, 4	3	1
3	0	30	1, 2, 4	0	1
4	9	19	Total ♂♂	269	

Table 12 shows the crossing over from sc-4/sc-8. Since one type of single crossover is practically lethal, the simplest way of calculating crossing over seems to be to use in each case only the larger of the two contrary classes. If this is done the values become: total 201; *y-f*, 19.9; *f-v*, 17.9; *v-cv*, 21.4; *cv*-w^a, 9.0. These values are, as expected, not different from those for sc-8/sc-8 or sc-8/Df sc-8. Classification for sc^8 was not attempted. The *y* males were typical sc^4; the *y* w^a were nearly wild-type for bristles, carrying both sc^4 and sc^8.

Both of the crossovers recovered from sc-4/sc-8 have been tested for crossing over and disjunction, and have given the expected results. The one with the left end of In sc-8 (the sc deficiency) when tested against a normal chromosome gave the following results: Df (sc) *f*/*y* w^a ♀ × various males: 453 regular ♀ ♀, no exceptions; 337 *y* w^a ♂ ♂, 7 *sc* (extreme) *f* ♂ ♂, 17 *y* w^a *f* ♂ ♂, 1 extreme *sc* ♂, 1 *y* ♂, 47 exceptional ♂ ♂. (In some cases sc^1, sc^{10}, or *slv* were used instead of *y*.) Calculating the frequency of exceptional males by doubling the *y* class (since the deficiency is nearly lethal), there were 47/767 or 6.1 percent exceptions. In other words this chromosome gives results both as to crossing over and as to disjunction comparable to those shown by the inversions from which it was derived. This result is confirmed by a small series in which the crossover was tested against In sc-8. Here there was about 32 percent crossing over between *sc* and *f*, and only 0.7 percent exceptional males were produced.

The other crossover (*sc* duplication, *bb* deficiency) behaves similarly. Females of the constitution *y* w^a *cv* Df(bb)//*f* gave 347 regular sons (of which 10 were crossovers, 3 clearly doubles and the others presumably so) and 11 exceptional sons. The totals for all experiments of this type show 12/391 = 3.1 percent exceptions. This same chromosome was also tested against In sc-4. There resulted 826 regular ♀ ♀, no exceptional ♀, 657 regular ♂ ♂, 4 exceptional ♂ ♂. Among the regular males crossing over could be checked, and the following values were obtained: *sc*-w^a, 0.5; w^a-*cv*, 7.0; *cv-v*, 18.3; *v-f*, 21.3. Here again the crossover, as expected, behaves much like the inversions from which it was derived.

Inversion sc-4/Inversion ClB

The ClB inversion lies wholly within that of sc-4. ClB/+ gives very few crossovers, so that doubles would be expected to be rare here, and none was found. Of the singles within the common inverted region, one type should be a deficiency for *slv-ec* and a duplication for *fu;* the other should be a duplication for *slv-ec*, and a deficiency for *fu*. Neither was obtained; evidently both are inviable in males and also in heterozygous females.

From ClB (*sc v B*)/*y* sc^4 *cv f* ♀ × y^2 *cv v f* ♂ were obtained 254 *v B* ♀ ♀, 238 *y cv f* ♀ ♀, 2 *sc B* (exceptional) ♀ ♀, 182 *y sc cv f* ♂ ♂, 1 y^2 *cv v* *f* (exceptional) ♂. An XXY ♀ of the same constitution gave 27 *v B* ♀ ♀, 33 *y cv f* ♀ ♀, 61 *sc B* ♀ ♀, 24 *y sc cv f* ♂ ♂ and 52 y^2 *cv v f* ♂ ♂.

Inversion sc-4/Inversion dl-49

Scute-4 inversion includes the segment from just to the right of *sc* to between *cr* and *bb*, the greater portion of the chromosome (fig. 1). Delta-49 extends from between *rb* and *cv* to between *fw* and *f* and is consequently entirely included within the sc-4 inversion. From the cross *y* sc-4/dl-49 *y-Hw* cm^2 m^2 g^3 × *t v m* g^2, 1,752 males were recorded, all non-crossovers. This result might have been expected from our knowledge of the behavior of females heterozygous for each of these inversions separately. Doubles within the sc-4 inversion and outside In dl-49 either do not occur or are very rare. Since heterozygous dl-49 gives single crossovers within the inversion (p. 587), it is probable that in the sc-4—dl-49 combination some singles occur in the inverted segment common to the two inversions. Such crossovers result in either a *slv-rb* duplication and *f-cr* deficiency or the complementary deficiency-duplication. Both of these products would be expected to be inviable.

XX females heterozygous for In sc-4 and In dl-49 give few or no exceptional daughters and a frequency of exceptional sons not significantly higher than normal.

Inversion sc-4/Inversion y-4

Inversion sc-4 runs from a point between *sc* and *slv* to a point between *cr* and *bb;* In y-4 from a point near *y* (to the left of *ac*) to a point between *fu* and *cr*. One of the single crossovers is deficient for *y* (?) *ac-sc* and *cr;* the other is a duplication for both these sections. The former is lethal in males but survives occasionally in heterozygous females as a minute-bristled type. The latter (duplication) crossover is viable in offspring of both sexes.

Owing to the presence of *y* in both inverted chromosomes and of an extreme *sc* allelomorph in sc-4, the tests for deficiencies for these two loci are inconclusive. Fully satisfactory tests have shown, however, that the minute crossover is deficient for the loci *ac* and *cr*, not for *rst*, *pn*, *sy*, nor for *od*. The other (duplication) crossover showed no deficiency effects for any of these loci. That is, the inversions differ with respect to *ac* and *cr;* In sc-4 was shown above to include *cr* but not *ac*. Hence y-4 includes *ac* but not *cr*. Both include *rst* and *pn*, as follows from this analysis and from the direct test. In sc-4 includes *sy* and *od;* therefore In y-4 has its right break between these two and *cr;* other data show that it is also to the right of *fu* which is 0.3 units to the right of *od* and *sy*.

The crossing over in In sc-4/In y-4 is similar to that in In sc-4/In sc-8. Here again one single crossover class of males dies, and the data have been treated by using the larger member of each pair of contrary classes. The results are then: total 433; *sc-f*, 20.3; *f-v*, 7.9; *v-cv*, 11.3; *cv*-w^a, 10.2; w^a-*sc*, 1.2.

The w^a *sc* value appears here and not in the corresponding series for In sc-4/In sc-8 because In y-4 carries a *sc* gene that is clearly dominant to sc^4, whereas the allelomorph present in In sc-8 gives with sc^4 a variable type not always clearly separable from sc^4.

Data from XXY In sc-4/In y-4 show the same type of crossing over and roughly the same amount; the experiments however include too few flies to be valuable for detailed comparisons.

Inversion sc-7/Inversion sc-8

This combination of inversions is essentially the same as sc-4/sc-7 already discussed. Females heterozygous for the two inversions give progeny carrying chromosomes derived by crossing over within the common inverted segment. As in the case of the sc-4/sc-7 both of these crossovers are inviable. Doubles within the sc-8 inversion but outside the short sc-7 inversion are recovered.

From the cross sc-8 w^a/sc-8 *v*/Y by various males the following offspring were recovered:

Regular	♀♀	589	
Exceptional	♀♀	70	
Exceptional	♂♂	103	
Regular	♂♂		
0		247	246
2-(3)		11	10

In addition there was one exceptional male expected to be y^2 *cv v B* which did not show *y* but which did show a hairy wing effect. This male was sterile. Evidently he carried a duplication for *y*.

From crosses of sc-7/sc-8 to T 1, 2–7 or T 1, 3–3 males (as in the case of sc-4/sc-7) males were recovered which carried the crossover deficient for the long segment from the right break in sc-7 to *bb*. These were enabled to survive by the proximal X segment from the translocation males. Such males differ from those obtained from sc-4/sc-7 (fig. 2) in not carrying a *bb* duplication and in carrying a duplication for the *sc* locus. From the cross sc-8 w^a *cv v f*/sc-7 w^a fa^2 *sn v* by translocation males the following flies were recorded:

		♂♂ T 1,2-7	♂♂ T 1,3-3
Regular females		844	867
matroclinous	♀♀	1	0
v minute	♀♀	0	1
Patroclinous	♂♂	4	5
Regular	♂♂	796	728
sc w^a	♂♂	17	19
sc w^a *fa*	♂♂	0	1

The *v* minute female recorded above carried the long crossover chromosome, tandem attached-X chromosomes deficient for the common inverted segment of the parent inversions. This deficiency was partly covered by the distal X segment from the translocation.

The *sc* w^a and *sc* w^a *fa* males carry the short crossover chromosome. The actual frequency was 2.1 and 2.7 percent of regular males. These males carry both the sc^7 and sc^8 genes and were intermediate between sc^7 and sc^8 males for the scute character. Their wings were spread. All of them that were tested (seven) were sterile as expected and were presumably XO.

Assuming that the translocation males produce four types of gametes in equal numbers, and that the above mentioned males have normal viability the true percentages of crossovers in the common inverted segment would be obtained by doubling the crossover male classes (the contrary crossover is not recovered) which would give values of 4.1 and 5.2 percent. Since these males are almost certainly of lower viability than the regular males, these percentages represent minimum values. The real value is probably considerably higher.

Inversion sc-7/Inversion ClB

These two inversions overlap in the rather short region from *bi* to near *cv*. Singles would be expected to be rare within this region and would be inviable. None was recovered. From the behavior of each when heterozygous for a normal chromosome it is inferred that few crossovers of any kind would be recovered. In fact, sc-7 w^a/ClB *sc v B* females gave 327 regular sons with one crossover which was sc^7 w^a *v* that is, a double within that part of In ClB not common to In sc-7. Similar XXY females gave 270 regular males with no crossovers.

Inversion sc-7/Inversion dl-49

The right break in In sc-7 is near *cv*, the left break in dl-49 between *rb* and *cv*. In the absence of more accurate information we cannot say definitely whether or not these two inversions overlap although it seems more probable that there is a short overlapping segment. The data from sc-7 w^a/dl-49 cm^2 XX females by *w B* males are limited; 157 males show no crossovers but the region to the right of the dl-49 inversion where one might expect a low frequency of crossovers is not under control. From XXY mothers, similarly marked, 206 non-crossover males were recorded.

Inversion sc-7/Inversion Df(bb)

In the combination of In sc-7 and In Df(bb) there is probably a short overlapping segment between *rb* and *cv*. From the cross sc-7 w^a/*y* sl^2 Df (bb)-× *sc ec ct t* g^2 *sl*, 1651 regular males were recorded of which 19 or 1.15 percent were apparent single crossovers between w^a and *sl*. Presumably all of these were actually doubles with the second crossover in the uncontrolled region between *sl* and *bb*. Several sc-7 w^a sl^2 males and *sc sl* females were tested and found not to carry Df (bb) showing that they were double crossovers. Presumably an appreciable number of undetected double crossovers occurred in the rather long unmarked region between the right end of In sc-7 and the locus of *sl*.

In the above cross 22 patroclinous males were recorded. Exceptional females could not be distinguished from one crossover class but since there were only 18 females in this class and 12 in the contrary crossover class, the number of exceptional females could not have been large.

Inversion sc-8/Inversion ClB

Inversion ClB is wholly within the limits of In sc-8, and few or no recovered crossovers of any kind are to be expected—the case being very similar to that of In sc-4/In ClB. In fact none was obtained among 21 regular sons of XX females or 160 sons from XXY females.

Inversion sc-8/Inversion dl-49

This combination is essentially similar to the combination of In sc-4 and In dl-49 already considered; the discussion given there applies in the present combination. From the cross sc-8 w^a/dl-49 cm^2 by *w B*, 1742 non-crossover regular males were recorded and 6 patroclinous males. From 5 additional cultures not recorded in detail, 2 apparent w^a *cm* males were obtained. These proved, on testing, to be the result of mutation of an eye color gene in the dl-49 chromosome rather than of crossing over. The locus of the mutation was not determined. From XXY females of the above constitution mated to *w B* males 576 non-crossover regular males and 444 patroclinous males were recorded.

Stone and Thomas (1935) also studied this combination. They obtained one double crossover (outside of the dl-49 inversion, inside of the sc-8 one) in experiments carried out at 30°C.

Inversion sc-8/Inversion y-4

Inversion y-4 extends to the left further than In sc-8 by the locus of *ac* (and *y*?); In sc-8 extends further to the right by the *cr- bb* section. No crossovers are to be expected outside the inversions; of the singles within the common inverted region, one is a duplication for *ac* (and *y*?) and for *cr-bb;* the other is a deficiency for both these sections. The latter is lethal in males, the former survives; in heterozygous females the former (duplication) is fully viable, the latter gives a minute-bristled individual that has reduced viability.

Tests against recessives show that the "minute" crossover is deficient for *ac* and *cr*, not for *slv;* the other one (duplication) is deficient for none of these loci. Therefore the inversions differ in that one includes *cr*, the other does not; likewise they differ for *ac;* both or neither include *slv*. These results are in agreement with the conclusions from sc-4/sc-8 and sc-4/y-4, which show that *slv* is included in both, *cr* in sc-8 but not in y-4, *ac* in y-4 but not in sc-8.

The crossing over tests for this combination show results similar to those from sc-4/y-4 but they are too scanty to permit detailed comparisons.

The duplication crossover, tested against a normal chromosome gave no exceptions among 314 daughters, 18 among 332 sons (5.4 percent). These values, as expected, are comparable to those from the In sc-4—In sc-8 crossovers.

Inversion ClB/Inversion dl-49

The delta-49 inversion is entirely included within the ClB inversion. From the cross ClB *sc v sl B*/dl-49 cm^2 bb^x × *sc cv v f cr*, 629 regular non-crossover males (cm^2) and 2 patroclinous males were obtained. Among 1266 females, 3 (0.24 per cent) were crossovers between *sc* and the left break of ClB; one was of the constitution *sc B* presumably a primary exception equational for the scute gene. XXY females of this combination were not studied.

Single crossovers in the inverted segment common to ClB and dl-49 presumably occur but are evidently inviable.

Inversion ClB/Inversion y-4

The right inversion points here are very similar, differing only in that In y-4 includes *fu*, In ClB does not. At the left In y-4 is considerably longer. Crossing over might occur between the inversions and the spindle attachment, but tests have not been made. Of the singles within the common inverted region, one gives a long deficiency for (*y*?) *ac* to *ec*, and a short duplication for *fu*, the other has the corresponding duplication and deficiency. Both crossovers are lethal in males. The one with the long (*ac-ec*) deficiency is also lethal in heterozygous females; the other is viable. This latter crossover usually carries the *B* gene of ClB, and in that case the resulting *B*/+ females have very narrow bar eyes similar to those of *B*/*B*. They also have irregularly arranged acrostical hairs, and their wings are slightly reduced in size and are less convex than is normal on the posterior margin. Tests of this crossover chromosome show that it carries a deficiency for *fu*, not for *y*, *ac*, *br*, *w*, *ec*, *f*, *vb*, *sy*, *od*, *cr*. The negative results could all have been predicted from conclusions already established in this paper; the positive case constitutes our proof that *fu* is in the inverted section of y-4, not in that of ClB.

From y-4 w^a/ClB *sc v B* only one of the 280 regular sons was a crossover. This one, y^4 w^a v, was a double within the common inverted region. The females from these same mothers (excluding the mating to *fu* ♂ because of the low viability of *fu*/Df) gave 218 broad bar non-crossover ♀ ♀, 237 not-bar non-crossover ♀ ♀ and 72 narrow bar crossover ♀ ♀. From the mating to *fu* ♂ one of the 6 *fu*/Df daughters was not-bar, and must have resulted from crossing over between *B* and the inversion point of ClB, a distance of less than two units on the standard map.

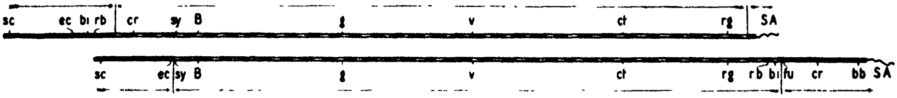


FIGURE 4.—In Df (bb) (above)/In ClB (below) Conjugation of the common inverted regions.

Inversion ClB/Inversion Df (bb)

The left break of ClB is between *bi* and *ec*, the right between *sy* and *fu*. The left break of Df (bb) is between *rb* and *rg*, the right between *cr* and *bb*. That these statements are correct will be shown below from studies of single crossovers between ClB and Df (bb). Both breaks of Df (bb) are to the right of those of ClB. Consequently one single crossover should give duplications for the *bi-rb* and *fu-cr* segments (fig. 4). The contrary crossover should give a deficiency for these two segments plus the deficiency for bobbed from the Df (bb) chromosome (fig. 4). Both of these crossovers are viable and can be recovered in heterozygous females. The duplication chromosome is viable in the male. Such males are small with wings having a less convex outer margin than normal and usually with one or more notches at the tips; they are sterile. Dissections by Professor DOBZHANSKY show that the testes are collapsed like those of very old males. Females heterozygous for the double deficiency survive as extreme minutes with wings of a characteristic shape. They have normal ovaries as shown by dissections made by Professor DOBZHANSKY but according to many tests are sterile. Males carrying this deficiency chromosome are inviable.

Tests of the deficiency-carrying crossover which gets the right end from Df (bb) and the left end from ClB give positive evidence that the *rb* and *cr* loci are absent. From crosses of ClB/Df (bb) to *bi* and *fu* males no deficiency heterozygotes were obtained. We conclude that both of these loci are included in the deficiency crossover. The results from ClB/y-4 establish this for *fu*. The number of flies examined was in each case adequate to have given many deficiency crossover females were they not inviable. Similar tests have shown that the *ec*, *rg*, *f*, *bb*, and *sy* loci are not included in these deficiencies. These results confirm the conclusion already drawn that the right break of ClB is between *sy* and *fu*. The left break must lie between *ec* and *bi*. These conclusions confirm and extend those of PAINTER (1934) derived from studies of salivary chromosomes. In a similar way it is clear that the left break of Df (bb) lies between *rb* and *rg* and that the right one lies to the right of carnation.

Frequency and distribution of single crossovers

Among 2010 regular females from ClB/Df (bb) mothers, 48 or 2.4 per cent carried the deficiency crossover. The contrary crossover could not be classified accurately in the females; if they were of equal frequency, the recovered single crossovers would be 4.8 percent of the total. Among 749 regular males, 26 or 3.5 percent carried the double duplication crossover. The contrary class dies but half the non crossovers likewise die because of the ClB lethal so that this value represents a direct measure of the frequency of singles. It is quite certain that both of the above values are much too low; the crossover-carrying individuals in both cases are of very poor viability and no precautions were taken to prevent overcrowding in the cultures.

It is of interest to determine how the single crossovers in the common inverted segment are distributed. In all experiments *v* was carried by the ClB chromosome. We can then separate crossovers in males into those which occurred between *rg* and *v* and those which occurred between *v* and *sy*. When this is done, the following results are obtained:

Crossover interval	Standard map lengths	Number of crossovers
rg to *v*	22+	111
v to *sy*	27.2	141

The ratios of standard map lengths of these intervals is 1:1.24−; that of singles within these regions 1:1.26. We can conclude that the distribution of single crossovers within the common inverted segment is approximately normal.

Crossing over in heterozygotes for the duplication crossover

Crossing over in females heterozygous for the Df (bb)-ClB crossover has been studied in two experiments. Only one of these is reported here. The other, although involving larger numbers of individuals, is not as well controlled for crossing over in different regions. The data from females carrying the duplication chromosome and a bobbed deficiency chromosome are given in table 13.

TABLE 13

Data from the cross y^2 *f v* dup/*sc* g^2 ct^6 *Df (bb)* ♀♀ by *w B* ♂♂. *The females of this experiment were sisters of those used in the experiment summarized in Table 10, homozygous Df (bb) of the same constitution.*

		B ♀♀	1471		
		+ ♀♀	0		
		w B ♂♂	5		
*Regular ♂♂					
0	91	411	1–5	0	6
1	99	14	2–3	0	2
2	60	14	2–4	0	3
3	55	7	3–4	0	1
4	31	6	3–5	0	1
5	7	0	1–3–4	1	0
1–2	0	8	1–2–4	1	0
1–3	3	14			
1–4	0	8	Total	844	

* In each case the smaller of the two contrary classes represents the males carrying the duplication.

In comparison with the Df (bb) chromosome, the double duplication crossover chromosome carries one net duplication, namely a segment including *rb-bi-f-cr* from the ClB chromosome. This segment together with the bb segment is simply added to the Df (bb) chromosome which of course carries the *bi-rb-cv-f* segment (no difference in arrangement) at the left end. The data of table 13 show the following crossover values as measured in the classes not carrying the duplication:

y-f	19.4
f-g	10.6
g-v	10.5
v-ct	6.4
ct-dup	2.1

For these same regions in homozygous Df (bb) sister females with a Y chromosome the values for the first four regions above (Table 13) are 37.1, 11.6, 7.7, 5.7 respectively. The fifth region cannot be measured in Df (bb)/Df (bb); its standard map length is 10 units.

It is clear from the above that the duplication crossover chromosome crosses over freely with Df(bb), one of the inversions from which it was derived. As compared with Df(bb)/Df(bb)/Y, crossing over is reduced in the *y-f* interval but is the same in the other intervals which can be compared. The reduction in the *y-f* interval is presumably the result of the duplication which is of course homologous with a segment included in the *y-f* interval.

Inversion dl-49/Inversion y-4

From the cross y-4 w^a/dl-49 *y Hw* m^2 g^3 ♀ ♀ by *t v m g* ♂ ♂, 1506 regular sons showed no crossovers. There were two patroclinous males. There were 1,651 regular females, all non-crossovers, and five exceptional females, four from 1 of the nine cultures.

From a cross of XXY females of the above constitution with *w* or *w B* males, 428 regular non-crossover, 487 patroclinous males, 446 regular females and 506 exceptional females were recorded.

It is clear that recoverable crossovers are practically absent in females of this combination, unless they occur between the right break of y-4 inversion and the spindle attachment. Data from attached-X females heterozygous for y-4 (p. 564) indicate that singles in this interval are very rare. Single crossovers in the segment common to the two inversions, presumably occur and are lethal both in heterozygous females and in males.

Inversion y-4/Inversion Df (bb)

The location of the breaks in both y-4 and Df(bb) have already been discussed; both breaks in Df (bb) are to the right of those of y-4. Consequently single crossovers in the common inverted segment of these two inversions will give either a double duplication or a double deficiency. The latter is inviable both in males and in heterozygous females; the former is viable in females heterozygous for a normal chromosome. Such a female usually has stubby outer verticals, disarranged scutellars, and outer wing margins less convex than normal. If such a duplication female is heterozygous for *B*, the eyes are usually as narrow as those of a female homozygous for *B*. These crossover females are fertile but produce very few offspring. Their viability is good considering the number of loci carried in the two duplications.

From the cross y-4 w^a *cv v* s^2/*sc* sl^2 Df(bb) by *sc B* males there were recorded one exceptional female (+), 330 regular ♀ ♀ (*B* and *sc B*), 4 *sc B* ♂ ♂, 123 *sc sl* ♂ ♂, 85 *y* w^a *cv v s* ♂ ♂ and 38 duplication ♀ ♀. The frequency of crossover females in percent of regular females is 10.7. From sister females of those used in the cross above, but XXY in constitution, mated to *sc B* males, the following offspring were obtained: 264 + ♀ ♀, 390 *B* ♀ ♀, 236 *sc B* ♀ ♀, 272 *sc B* ♂ ♂, 159 *sc sl* ♂ ♂, 198 *y* w^a *sv v s* ♂ ♂, and 144 duplication ♂♂. Here duplication females constitute 18.7 percent of the regular females. Since only one crossover is recovered here the true percentage of singles from these data will be 18.7 for XX and 19.8 (corrected) for XXY. The frequencies are not significantly different.

Since females heterozygous for the duplication crossover produce very few offspring, few studies of them were made. It is known that crossing over between the duplication chromosome and a normal chromosome is very low and that a few patroclinous males are produced. These results are expected since the crossover chromosome is in effect an inversion plus an intercalated duplication.

NON-DISJUNCTION

Table 14 is a summary of the available data on the production of matroclinous females and patroclinous males.

In many of these experiments the exceptional females could not be distinguished and only the males are recorded, in others the male exceptions were known to have very low viability and only the females are recorded. It follows that the numbers of individuals in the two sexes from a given combination are often not comparable. ClB and Df(sc-8) are lethal in the males; accordingly in all series involving these the observed number of regular males has been doubled in calculating the recorded total, a point to be remembered in judging the significance of the values given. In many of the combinations of two inversions, single crossovers between the two inversions occur; some of these are lethal and others have reduced viability. No corrections have been made for this; therefore in several of these cases if is certain that the totals are too low and the percentages of exceptions too high.

In the case of ClB/+ we have added our own data to those recorded by GERSHENSON (1935) though in the XXY experiments we obtained somewhat higher values than he records. We have excluded the males from his XX experiment in which the father was bb^l, since the exceptions (having no Y) would be inviable. We have also excluded one unexplained XX culture of our own that gave 9 exceptional females to 223 regulars and 11 exceptional males to 133 regulars. We have observed in some other combinations a suspiciously high frequency of cultures that gave more than one exception when others of the same constitution gave none. In no case were the resulting frequencies high enough to be interpreted as due to the presence of an unsuspected Y. The frequencies are about those that result from the presence of a short duplication carrying the X spindle attachment, but we have not studied the descendants of such females with this possibility in mind.

The results of STONE and THOMAS (1935) for sc-8/+ and dl-49/+ have not been included in the table.

TABLE 14

Summary of non-disjunction data.

	XX MOTHERS						XXY MOTHERS					
	FEMALES			MALES			FEMALES			MALES		
	TOTAL	EXC.	% EXC.	TOTAL	EXC.	% EXC.	TOTAL	EXC.	% EXC	TOTAL	EXC.	% EXC.
sc-4/+	6287	1	0.02	5861	337	5.75	933	40	4.3	817	109	13.4
sc-4/sc-4	953	0	0.00	600	0	0.00						
sc-7/+	5386	5	0.09	4919	14	0.28	1518	195	12.9	1697	256	15.1
sc-7/sc-7	1610	0	0.00	1370	1	0.07						
sc-8/+	4703	1	0.02	5138	164	3.20	1310	130	9.9	1847	255	13.8
sc-8/sc-8	574	0	0.00	481	0	0.00	310	4	1.3	252	5	2.0
Df (sc^8)/+	641	2	0.31	1053	47	4.46	85	9	10.6	80	12	15.0
sc-8/Df (sc^8)	631	0	0.00	504	9	0.00						
ClB/+	5693	14	0.25	3438	16	0.47	7478	2729	36.6	7172	2712	37.8
dl-49/+	3238	0	0.00	3168	6	0.19	4355	1985	45.6	4145	1747	42.2
dl-49/dl-49							126	5	4.0	99	12	2.0
y-4/+	2007	1	0.05	2171	51	2.34	587	39	6.8	631	56	8.9
y-4/y-4	206	1	0.49	169	0	0.00						
Df (bb)/+	1244	0	0.00	3437	67	1.95	1877	255	13.6	1836	292	15.9
Df (bb)/Df (bb)							2338	69	2.9	2100	84	4.0
sc-4/sc-7	459	0	0.00	422	7	1.66	341	34	10.0	280	43	15.3
sc-4/sc-8	456	0	0.00	264	0	0.00						
sc-4/dl-49	2084	0	0.00	1755	3	0.17						
sc-4/y-4	1187	3	0.26	954	0	0.00	413	33	8.0	224	30	13.4
sc-7/sc-7	439	0	0.00	350	12	3.43	659	70	10.6	617	103	16.7
sc-7/Df (sc^8)	523	0	0.00	531	7	1.32						
sc-7/ClB	754	0	0.00	658	4	0.61	894	322	35.8	824	284	34.5
sc-7/dl-49	169	2	1.18	157	0	0.00	355	138	38.8	346	140	40.5
sc-7/y-4	479	0	0.00	297	1	0.34						
sc-7/Df (bb)				1673	22	1.31						
sc-8/dl-49	1959	5	0.26	1748	6	0.34	1146	541	47.3	1020	444	43.5
sc-8/y-4	316	0	0.00	240	1	0.42	906	70	7.7	968	134	13.8
ClB/dl-49	1270	4	0.32	1260	2	0.16						
ClB/y-4				561	1	0.18	101	4	4.0	57	5	8.8
ClB/Df (bb)	1557	2	0.13	1200	6	0.50	1622	520	32.0	1351	561	41.5
dl-49/y-4	1656	5	0.32	1508	2	0.13	952	506	53.2	915	487	53.2
y-4/Df (bb)	370	1	0.27	194	4	2.06	1034	264	25.5	629	272	43.2

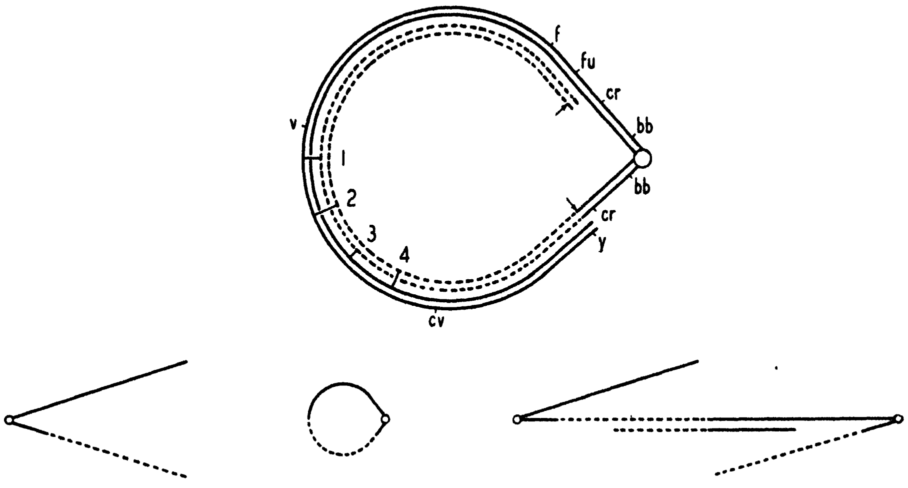
PRESENCE OF SINGLE CROSSOVERS

Representing a normal X chromosome schematically as *B C D E* a and an homologous chromosome with the *CD* segment inverted as *B D C E* a (a in both cases representing the spindle attachment), then single crossovers in the *CD* segment will give the products (1) *B C D B* (duplication for *B*, deficiency for *E*, and having no spindle attachment) and (2) a *E C D E* a (deficiency for *B*, duplication for *E*, and having two spindle attachments). Product (1) would be expected to be lost because of its lack of a spindle attachment. Product (2), because of its two spindle attachments, should form a tie between the two poles of the first meiotic division. It is known from the cytological studies of McClintock (1931, 1933) on Zea, Mather (1934) on Vicia, Stone (1933) on Tulipa, and Smith (1935) on Trillium that, for these plants, single crossovers do occur between segments relatively inverted and that the results are as described above. In order to understand the mechanism of disjunction in inversion heterozygotes in Drosophila it is essential to know whether such crossovers occur in this organism and, if so, with what frequency. The most direct method of answering these questions, namely, cytological examination as used in the cases cited above, is very difficult in the case of oögenesis in *Drosophila melanogaster*. We have resorted to less direct genetic methods.

From the data already presented on single crossing over in combinations of two overlapping inversions it seems highly probable that single crossovers occur within the inverted segment in females heterozygous for a single inversion. Inversion scute-8 represents an inversion of the entire X chromosome with the exception of the *y* and *ac* loci and the spindle attachment. It can be considered as representing essentially a transfer of the spindle attachment from the right to the left end of the chromosome.

As regards crossing over it should behave essentially like a normal chromosome. In the heterozygote of sc-8 and sc-4 crossing over is practically normal as has already been shown. We can therefore argue that in the heterozygote sc-4/+, single crossovers should be of approximately normal frequency. Similarly in the combination sc-7/sc-8 we have shown that single crossing over occurs in the common inverted segment. From this we can conclude that single crossing over occurs in the inverted segment of sc-7/+. Here the data from the combination sc-7/sc-8 and also from sc-4/sc-7 suggest that the frequency is reduced below that for the sc-7 inverted segment normally arranged. The same general kind of an argument can be made for several of the other combinations considered in the previous section.

In a female heterozygous for a single X chromosome inversion, crossing over can be more or less directly measured by using attached-X females, (see also Sidoroff, Sokolov, and Trofimov 1935). By selecting the appropriate crossover from a triploid of the constitution y-4/$\widehat{XX}$ we obtained an attached-X female heterozygous for the y-4 inversion. In such a female, exchange in the inverted segment will give either (1) a closed chromosome carrying a duplication for *cr-bb* and a deficiency for the small segment to the left of *y*, or (2) a chromatid with two spindle attachments plus a chromatid with none (fig. 5). The Y chromosome that is usually present disjoins from the attached-X chromosomes at the first division. The results described above and shown in figure 5 take place during the second division. The types and relative frequencies of gametes expected following single and double exchange are summarized in table 15. Data from the cross y^4cr/y^2v for attached-X females mated to *t v f* males are given in table 16.



Second division following 1 or 4

Second division following 2 or 3

FIGURE 5.—Attached-X, heterozygous for In y-4. Above, conjugation of inverted section. Below, chromatids resulting from the indicated single exchanges. Chromatid with no spindle attachment omitted in lower left.

TABLE 15

EXCHANGE	$\widehat{XX}$ GAMETES				X^c GAMETES	SECOND DIVISIONS WITH CHROMATID TIE	Y GAMETES
	NON-CROSSOVER	RECIPROCAL	EQUATIONAL-a	EQUATIONAL-b			
None	1						1
Single	1				1	2	4
Double* 2-s	1	1	1	1			4
3-s a					2	2	4
3-s b			1	1	2		4
4-s						4	4
Total Doubles	1	1	2	2	4	6	16

* Double exchanges are designated by the number of strands that undergo crossing over. Thus, in a 2-strand (2-s) double exchange, the same two strands cross over at the two levels.

The daughters showing *y* and *cr* carry attached-X chromosomes. Those which show neither *y* nor *cr* carry the closed chromosome derived by single crossing over in the inversion. From the fact that the sex ratio approaches 2 males to 1 female we infer that those cases in which a double spindle attachment arises by crossing over within the inversion result in lethal eggs.

TABLE 16

Progeny of the cross $y^4cr/y^2v\ f\ cr$ attached-X females by $t\ v\ f$ males.
Four egg-laying periods; 3, 2, 2 and 3 days.

$\widehat{XX}$ FEMALES		$+/X^c$ FEMALES		MALES	
y^2cr	304	f	137	$t\ v\ f$	1098
$y^2v\ cr$	11	$v\ f$	197	$y\ cr$	1
$y^2v\ f\ cr$	1	v	3		
Total	316		337		

In such cases the X chromosomes are not simply eliminated for we should then expect a ratio of 2+ males to 1 female. Males carrying the closed chromosome are almost completely inviable. The *y cr* male recorded in table 16 apparently carried such a chromosome. This male had narrow wings, other characteristics of duplication-carrying males, was *y* (not y^2) and was sterile. We have not made a cytological study of this closed-X; this has been done by SIDOROV, SOKOLOV, and TROFIMOV (1935) for the closed-X they obtained in the same way (using an unspecified inversion). They have published a drawing of one metaphase plate showing the closed-X.

From the data given in table 16 and the information summarized in table 15, we can make an approximate calculation of the frequencies of single and double exchanges within the inverted segment. Doubles are measured by equationals for genes within the inverted segment (*v* and *f*). Since equationals for one chromosome only are detected, there is one chance in eight of detecting a crossover from a double exchange tetrad. Thus the twelve equationals indicate that there were actually 96 double exchange tetrads. Since a part of the females are eliminated, a corrected total must be used and the most direct way of getting this is to double the number of males. This gives 2196 as a corrected total. The frequency of double exchanges is then 4.4 percent. Using the information in table 15 we can subtract from the observed numbers of individuals those which carry products of double exchange. The remainder should give a measure of single exchanges. Single crossovers are directly recovered as closed chromosomes. The frequency of single exchanges is also measured by the deficiency of females as compared with males. The average of these two measures of single exchange is 90.8 percent (assuming 50 percent as the value given by recovered closed chromosomes). The summary of the above operations is as follows:

	$\widehat{XX}$ females	X/X^c females	males	Excess of males over females
Observed	316	337	1098	445
Double exchange (4.4%)	36	24		36
Remainder	280	313		409
Single exchange (90.8%)	298.7	248.4		454.9

The two measures of single exchange agree with one another only approximately. However it is quite clear that the frequency of single exchange is high and approaches the frequency characteristic of the segment normally arranged. The distribution of the single crossovers in the inversion heterozygote is indicated by the data, and for the regions *y-v* and *v-f*, is approximately the same as that for normal chromosomes.

Crossing over in the segment between the right break of the inversion and the spindle attachment is very low as indicated by the low frequency of forked equationals. The one y^2 *v f c r* female recorded in table 16 is assumed to have resulted from double exchange within the inverted segment rather than from single exchange to the right of it.

In the case of $\widehat{XX}$ females heterozygous for short inversions, the closed chromosomes resulting from crossing over within the inverted segment are inviable in heterozygous condition and so cannot be recovered unless the duplications and deficiencies are compensated for in some way. However it is clear from the above discussion of attached-X In y-4 heterozygotes that the distortion of the sex ratio in itself can be used as a measure of crossing over.

Early experiments with $\widehat{XX}$ females heterozygous for In sc-7 consistently gave an excess of males over females. The results of three such experiments are summarized in table 17. These experiments indicated relatively high frequencies of exchange in the inverted segment and approximately normal crossing over between the inversion and the spindle-attachment, the latter result being in substantial agreement with those from free-X In sc-7 heterozygotes (p. 557). Since no particular precautions were taken to insure that male and female offspring were of comparable viability in these experiments, stocks more satisfactory with respect to the mutant genes used were made up and the experiments repeated. To decrease viability differences, relatively short egg-laying periods were used.

The results are summarized in tables 18 and 19. These data are in agreement with those from the first experiments in showing that crossing over to the right of the inversion is about normal (the sc-7 chromosome of the females whose progeny are summarized in table 19 apparently carried a semi-lethal mutant of unknown origin and the raw data must be corrected accordingly). However these experiments indicate a lower exchange frequency within the inverted segment than did the earlier ones. The inversion involves a segment 13 to 20 map units long. The data of tables 18 and 19 give crossover values of 9 and 10 percent (one half exchange frequencies).

TABLE 17

Progeny of $\widehat{XX}$ females heterozygous for inversion sc-7 mated to various males.

CONSTITUTION OF DAUGHTERS*	CONSTITUTION OF MOTHERS		
	$\frac{sc\text{-}7\ w^c\ v\ f}{y^2\ w^a\ ec\ f}$	$\frac{sc\text{-}7\ w^a\ f}{y^2\ w^a\ ec\ f}$	$\frac{sc\text{-}7\ w^a\ v\ B}{y^2\ w^a\ ec\ f}$
+	267	206	155 (*B*/+)
sc-7	6	45	10 (*B*/+)
y^2 *ec*	53	24	18 (*B*/+)
sc-7 *v*	53		14 (*B*/+)
y^2 *ec f*			4
sc-7 *v*, *B*/*B*			5
B/*B*			2
f			1
y^2 *ec*, *B*/*B*			1
Total females	379	275	210
Corrected total females†	385	296	216
Total males	493	483	309
Exchange (%)	29.2	51.6	40.1

* Constitutions are given only with respect to genes heterozygous in the mother.

† Corrected total females obtained by adding to non-crossover phenotype, twice the number of equationals for the sc-7. The correction is for the indicated lower viability of equationals for y^2.

Experiments were made with In dl-49 using the same technique as for the later experiments with In sc-7. The results of two experiments with controls are summarized in table 20. The extent of the distortion of the sex ratio in the two series is not the same. The control experiments indicate that the difference is due to the difference in relative vaibility of the two kinds of males used. Making appropriate corrections of the number of males, the two series indicate exchange values for the inverted segment of 11.5 and 12.5 percent. Exchange in the segment between the right inversion break and the spindle attachment is measured by equationals for genes within the inversion limits. The values indicated are 3.8 and 10.4 percent for the two series. The cause of the rather large difference is not known. Exchange in the segment to the left of the inversion is measured by equationals for *y* and by equationals for the genes *v* which are not equational for *y*. About all that can be said about the exchange frequency for this terminal segment is that it is low (less than 2 percent).

TABLE 18

Progeny of the cross sc-7 w^acr/y^2ct v f cr attached-X females by wild type males. Egg-laying periods; 3, 2 and 2 days.

FEMALES								TOTAL*	MALES	PERCENT EXCHANGE
cr	sc-7 w^a*cr*	*y ct cr*	*y ct v cr*	*y ct v f cr*	*f cr*	*v f cr*	*v cr*			
629	124	27	79	45	2	3	1	937	1088	18.5

* Total corrected for indicated lower viability of sc-7 w^a

TABLE 19

Progeny of the cross sc-7w^a/y^2v f cr attached-X females by wild type males. Egg-laying periods 3, 2 and 2 days.

FEMALES										TOTAL*	MALES	PERCENT EXCHANGE
+	sc-7w^a	*y*	*y v*	*y v f*	*y v f cr*	*v f*	*f cr*	*f*	*v*			
393	2	14	66	8	1	1	1	1	1	575	679	20.4

* Total corrected as in table 18.

From the evidence considered above it is clear that single crossovers do occur between segments of chromosomes inverted relative to one another. The frequency of such crossovers evidently depends on the length of the

TABLE 20

Progeny of the cross dl-49 cm^2/y^2 v f cr attached-X females×Bar and + males and from control crosses of y^2 v f cr/y^2 v f cr attached-X females by Bar and +males. In each case, three egg-laying periods; 3, 2 and 2 days.

PROGENY	INVERSION		CONTROL	
	XB	X+	XB	X+
+ ♀♀	984	1672		
cm ♀♀	11	46		
y ♀♀	0	1		
yv ♀♀	2	10		
y v f ♀♀	4	28		
y v f cr ♀♀	5	18		
v f cr ♀♀	0	1		
Total ♀♀	1006	1776	590	841
♂♂	1004 (1101)*	2076 (1960)*	538	891

* Numbers in parentheses are corrected totals obtained by multiplying the observed numbers of males by the ratio of females to males in the appropriate control.

inverted segment and its position in the chromosome. These relations will be discussed in more detail in another connection (page 596). In any case we can say that long inversions such as In sc-8, In sc-4, and In y-4 show, with a normal chromosome, a high frequency of single crossing over between the inversion segments. These frequencies are of the same order of magnitude as those characteristic of these same segments arranged in the normal way.

EGG AND LARVAL-PUPAL MORTALITY

We have shown in the preceding section that single crossovers occur with a relatively high frequency in inversion heterozygotes. The question that we shall consider now is whether or not such crossovers result in inviable zygotes. This question can of course be directly answered by determining the amount of mortality in the progeny of heterozygotes for inversions known to give a high frequency of single crossing over within the inverted segment.

Technique

The method that we have used in determining the amount of mortality is essentially the same as that commonly used by other workers (e.g., L. V. MORGAN 1933). Certain modifications were found useful. Paper spoons have usually been used as containers for the medium on which the eggs are collected. They have two disadvantages: (1) the surface of the medium is usually not flat and (2) the depth of the medium varies which often results in drying out around the edges. To overcome these disadvantages small metal (nickel has been found satisfactory) containers about 38 mm long, 17 mm wide and 3 mm deep were made. A handle of the same material about 10 mm wide was soldered to the bottom so that it projected about 3 cm.

The standard cornmeal-molasses-agar medium with the addition of animal charcoal (to increase the contrast between eggs and medium) was liquified, pipetted into the containers and allowed to cool. The flat surface was then painted with a rather heavy yeast suspension. The addition of fermented banana, alcohol or wine was found to be of no advantage. A single female was allowed to deposit eggs on the medium for a period of 24 hours. The container was then removed from the vial and replaced with one containing fresh medium. After removing the container the food was removed from it with a strip of cardboard of appropriate size. The eggs, including those already hatched, were then counted and recorded. The food was placed in a vial at 25°C in a moist incubator for 28 hours after which time the unhatched eggs were counted. The food block was then placed in a standard half pint culture bottle and the flies allowed to develop to maturity. Unless care is taken to have the outside of the food container dry there is danger in error from eggs deposited on the sides of the container. The larvae from these eggs may hatch and crawl onto the food block. There is also some error in losing or killing a few larvae in handling the food blocks. The magnitude of these errors can be kept reasonably low with careful manipulation.

Our experience indicates that the percentage of egg mortality is dependent upon the genetic constitution of the mother as well as upon the genetic constitution of the eggs themselves. Thus females from inbred stocks or females homozygous for several recessive genes generally give relatively high mortality regardless of the type of males to which they are mated. Because of this fact, strictly comparable controls cannot be had. To minimize this "residual" egg mortality, crosses between more or less unrelated stocks were made wherever possible and the F_1 females from not overcrowded cultures were used in the egg-laying experiments. Normal controls more or less comparable in genetic constitution were run simultaneously with the experiments on inversion heterozygotes.

Results

The results of our experiments on In sc-4, In sc-8, In y-4, and In dl-49 heterozygotes are summarized in table 21. The answer to the question that we set out to study is quite clear: single crossovers do not give rise to inviable zygotes. In the cases of In sc-4, In sc-8 and In y-4 exchange is approximately normal in frequency. If the distribution of the four strands of a tetrad were random at meiosis, we should expect about half the products to be single crossovers or their equivalent. Since such crossovers are not recovered in the viable zygotes they would have to be eliminated as inviable zygotes. However, it is evident that inviable zygotes do not approach 50 per cent in frequency. Since these inversion heterozygotes produce an appreciable number of patroclinous males (about 3 to 5 percent of the viable zygotes) we know that there should be a corresponding frequency of inviable zygotes (no-X eggs fertilized by Y-carrying sperms). In these cases crossing over could be followed sufficiently well to know that the inversion heterozygotes were giving the usual results. In addition the frequency of patroclinous males was determined and found to be approximately "normal" for the inversion heterozygotes under consideration. When the data are considered in connection with the frequencies of patroclinous males produced (page 595) and with the controls, and when allowance is made for the difference in genetic constitution between inversion heterozygotes and controls, we can conclude that the only zygotes whose death is the direct result of the presence of the inversion in the parent females are those corresponding to patroclinous males and differentiated from them by the sperm.

Stone and Thomas (1935) have also published egg counts for In sc-8/+ and In dl-49/+. The mortality indicated is higher than in our data, as it is also for their controls. They have also not distinguished between egg mortality and larval-pupal mortality. It seems clear that the lowest adequately established mortality is the one that gives the best picture of the case; and it is to be noted also that Stone and Thomas conclude, as do we, that single crossovers are not responsible for any detectable portion of the observed mortality.

Table 21

Egg and larval-pupal mortality data for inversion heterozygotes.

MATING	TOTAL EGGS	INVIABLE	PERCENT INVIABLE	HATCHED EGGS	ADULT FLIES	PERCENT EMERGENCE OF HATCHED EGGS
y sc-4 $v\ f\ cr/+\times B$	2839	214	7.5	2625	2187	83.4
sc-8 $w^a\ cv\ v\ f/y^2\!-\!\times B$	4190	284	6.8	3906	3745	96.0
y^{2S}/B (control for above)$\times B$	3947	52	1.3	3895	3741	96.2
sc-8 $B/w^a\ v/Y\times y^{2S}$	1313	184	14.0	1129	990	87.6
$+/w^a\ v$ (control)$\times y^{2S}$	1018	26	2.5	992	991	99.9
dl-49/$+\times B$	1645	51	3.1	1594	1525	95.7
y-4/$v\ g^3\times w$	884	68	7.7	816	753	92.4
y-4/y-4$\times w$	570	113	19.8	457	375	82.2

Data from XXY In sc-8 heterozygotes are included in table 21. Here both egg and larval-pupal mortality is relatively high. This is of course expected from the fact that here secondary non-disjunction occurs with an appreciable frequency (page 582). The frequency of inviable eggs is of the same order of magnitude as that of exceptional males (YY or YX zygotes, depending upon the sperm) and larval-pupal mortality is of the same order of magnitude as exceptional females (XXX or XXY zygotes, depending upon the sperm).

In connection with studies of sister-strand crossing over, Schweitzer and Kaliss (1935) have made extensive determinations of egg mortality in inversion heterozygotes. Their results are in agreement with the conclusion we have drawn that single exchanges between inverted segments do not result in inviable zygotes.

THE MECHANISM OF DISJUNCTION IN INVERSION HETEROZYGOTES

It has been shown in preceding sections that single exchanges occur within the inverted segment of inversion heterozygotes, and further, that the crossover products of single exchange are not recovered and do not result in inviable zygotes. It is evident that we must assume that such single crossover chromatids are selectively eliminated during the meiotic process.

It has also been shown that X chromosome inversion heterozygotes give rise to patroclinous males among their progeny. We have implied that the frequency of such exceptional males is a function of double exchange.

The problem that we shall consider is how these two results, (1) elimination of single crossover chromatids and (2) the production of no-X eggs, are brought about. We know from cytological studies on plants (Zea, McClintock 1933; Tulipa, Stone 1933; Vicia, Mather 1934; Trillium, Smith 1935) that double spindle attachment chromatids resulting from crossing over between segments inverted with respect to one another produce chromatin ties between the two poles of the first meiotic division (or under certain conditions to be considered below, between poles of second meiotic spindles). Knowing that the four nuclei resulting from meiosis in the Drosophila egg lie approximately on a single straight line (Huettner 1924), we are prompted to propose the following scheme for the X chromosome of Drosophila.

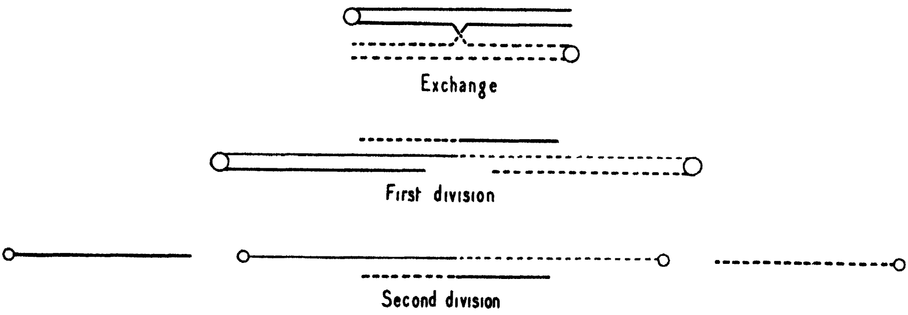


Figure 6.—Single exchange within a heterozygous inversion. The upper figure represents the two X's of a female in which one chromosome is practically wholly inverted. At the first meiotic division there results a chromatid tie; this leads to an orientation of the second division such that the two terminal nuclei receive only non-crossover chromatids; one of these is the egg nucleus. The result is the total loss of all single crossover chromatids to the polar body nuclei.

1. A single chromatid tie at the first meiotic division results in orientation of the spindle attachments in such a manner that only chromatids with a single spindle attachment get into the terminal nuclei, one of which will become the egg nucleus (Huettner 1924).
2. A double chromatid tie results in the formation of end nuclei with no X chromosome, and a no-X egg will result.

The behavior of various types of crossover tetrads expected according to this scheme is shown diagrammatically in figures 6 and 7.

As to the precise nature of the orientation of single exchange tetrads or their equivalent we have insufficient information; we know only the end result. It seems reasonable to suppose that the orienting influence of a double attachment chromatid is mechanical. However, we do not know whether or not such chromatids in Drosophila break during the division as they are known to do in the plants mentioned above. If they break, the orientation probably results from the retardation prior to breakage. Single crossover chromatids without spindle attachments are probably not included in either daughter nucleus but lost in the cytoplasm during division as is known to be the case in plants (McClintock 1933).

From the diagrammatic representation of the suggested scheme (figs. 6 and 7), it is evident that certain quantitative relations should hold. These should serve as tests of the assumptions we have made. The types and frequencies of gametes expected to result from non-, single, double, and triple exchange tetrads are summarized in table 22. It is seen that from double exchange tetrads, double crossover and no-X gametes are expected to occur in the ratio of 3:2. This 3:2 ratio was also found experimentally by Stone and Thomas (1935) for In sc-8 and for another long inversion that we have not studied. For the longer inversions some triple exchanges presumably occur and these give double crossover and no-X gametes in the ratio of 21:4. Since we have no way of measuring the relative frequencies

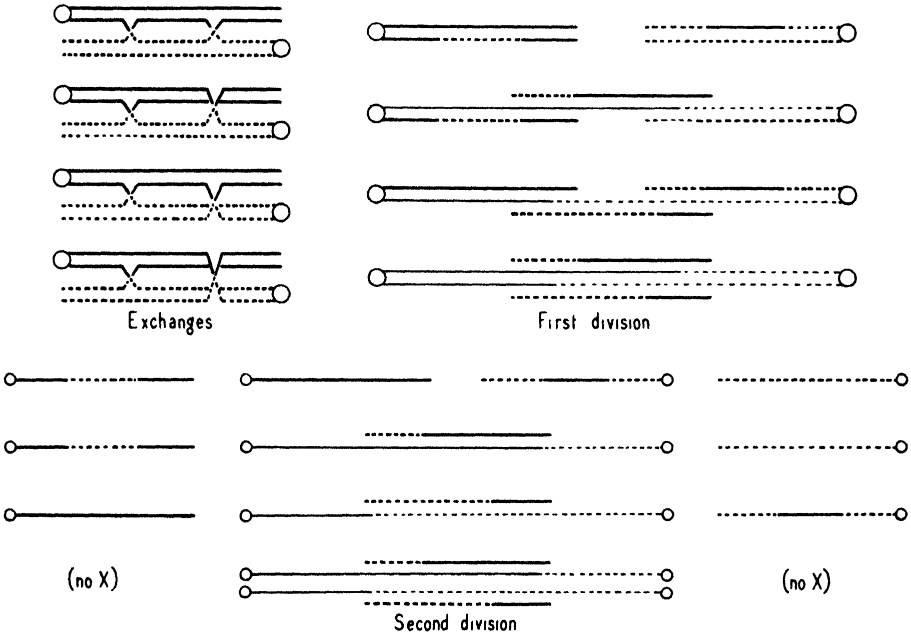


Figure 7.—The four possible types of double exchange within a heterozygous inversion. The two-strand exchange (upper row) leads to equal numbers of non-crossover and double crossover chromatids in the terminal nuclei, each of which will be the egg nucleus in half the cases. Three-strand doubles (second and third rows) result in chromatid ties at the first division, and also lead to equal numbers of non-crossover and double crossover chromatids in the egg nuclei Four-strand double exchange (fourth row) leads to a double tie at the first division, and to no-X egg nuclei.

of double and triple exchanges in inversion heterozygotes (because triple crossover chromatids must be eliminated) we cannot predict precisely what the ratio of double crossover to no-X eggs should be. However, if we make the assumption that the frequency of triple relative to double exchanges in inversion heterozygotes does not exceed that of normal X chromosomes (about 1:10), then the ratio of double crossover to no-X gametes should lie between 3:2 and 3.4:2.

The numerical data showing the relation of double crossovers to patroclinous males are given in table 23. Half the no-X eggs are lost (fertilization with Y sperm) and in the cases recorded in the table, half the double crossovers were not detected since only male offspring were used to measure crossing over. Hence the zygotic ratios expected are the same as the gametic ratios mentioned above and given in table 22. The observed relative frequencies approach rather closely the ratio 3:2. In no case is the deviation from 3:2 statistically significant. The totals approach very closely a

TABLE 22

Relative frequencies of types of gametes produced following single, double, and triple exchange within the inverted segment of an X chromosome tetrad.

EXCHANGE	DESIGNATION*	NON-CROSSOVER	DOUBLE CROSSOVER	NO-X EGG
None		1	0	0
Single		1	0	0
Double	2 (1)	1	1	
	3 (2)	2	2	
	4 (1)			2
	Total	3	3	2
Triple	2, 3, 3 (4)	4	4	
	2, 4, 4 (2)		4	
	2, 2, 2 (1)	2		
	3, 4, 3 (2)			4
	3, 2, 3 (2)	1	3	
	3, 3, 4 (4)		8	
	4, 2, 4 (1)		2	
	Total	7	21	4

* Doubles are designated by the number of strands involved in the two exchanges; triples in the same way by considering them as three doubles, taking successive exchanges a, b and c in combinations of two in the order a-b, a-c, and b-c, and in this case, disregarding the direction (a-b-c is equivalent to c-b-a). Relative frequencies of different types among doubles or among triples are indicated by numbers in parentheses.

The frequencies of gametes (totals of last three columns) must in each case be proportional to the frequencies of occurrence of the types of exchange (numbers in parentheses). This is true even in the case of two-strand doubles, where the potentially good chromatids are twice as numerous as in the case of three-strand doubles, since each tetrad gives rise only to a single gamete. The same principle applies also to triples.

ratio of 3:2; the actual observed small deviation is in the direction expected to result from triple exchanges. The inversion heterozygotes known to give approximately normal crossing over (sc-4, sc-8 and (Df sc^8) actually are the ones that give the higher ratios. The results expected on the proposed scheme of disjunction in inversion heterozygotes are thus in quantitative agreement with the experimental data.

TABLE 23

Relative frequencies with which double crossovers and patroclinous males are recovered from inversion heterozygotes. In all cases double crossovers as recovered in males only are recorded.

CONSTITUTION	DOUBLE CROSSOVER MALES	PATROCLINOUS MALES	CALCULATED (3:2)		ACTUAL RATIO
sc-4/+	108	63	102.6	68.4	3.4:2
sc-8/+	93	57	90.0	60.0	3.3:2
Df (sc-8)*/+	60	31	54.6	36.4	3.9:2
y-4/+	57	51	64.8	43.2	2.2:2
Df (bb)/+	93	66	95.4	63.6	2.8:2
sc-4/sc-7	11	7	10.8	7.2	3.1:2
	422	275	418.2	278.8	3.1:2

* Number of double crossovers corrected, owing to lethal nature of Df (sc-8)

The scheme proposed should enable one to predict quantitatively the results from closed-X heterozygotes. These have been studied by L. V. MORGAN (1933). Her results differ from those expected according to the scheme formulated from our knowledge of inversion heterozygotes in two important respects:

1. Among the progeny of X/X^c, X is recovered more frequently than X^c.

2. Egg mortality is too high relative to the frequency of recovered double crossovers.

The inequality of X and X^c among the progeny was ascribed by L. V. MORGAN to differential viability. The egg mortality data led her to conclude that single exchanges result in inviable zygotes. Fortunately a second closed-X chromosome was found by Mr. R. D. BOCHE of this laboratory. This closed-X has an advantage over the original X^c used in the experiments of L. V. MORGAN in that it has less effect on viability. We have made several experiments with X^{c-2} heterozygotes set up especially to test the scheme proposed in this paper. The results of these experiments are to be presented in another paper but we can say here that both discrepancies mentioned above appear now to be viability effects. The results obtained from X/X^{c-2} and X^{c-2}/X^{c-2} females are in as good agreement with those predicted from the assumptions we have made in this paper as could reasonably be expected.

We have pointed out that single exchange between two segments inverted relative to one another does not result in inviable zygotes. In the case of attached-X inversion heterozygotes we have pointed out that following certain types of single exchange a chromatid tie is formed during the second meiotic division. From the numerical data we concluded that the condition in which the X chromosome spindle attachment is tied to a spindle attachment in the nucleus lying next in line does result in an inviable egg. There is another case in which single exchange within the inverted segment should result in a chromatid tie at the second division. This is where a single exchange within the inversion is accompanied by a second exchange outside the inversion of such a nature that the two exchanges make a three-strand double exchange. SMITH (1935) has observed this result cytologically in Trillium. In the inversions dealt with in our experiments, lethal eggs from this source would be very infrequent except for the case of In sc-7. This inversion is the only one that we have used in which exchange in the heterozygote is frequent both inside and outside the inverted segment. Here, however, the mortality has not been studied.

EFFECTS OF INVERSIONS ON FREQUENCY OF CROSSING OVER

a. Homozygous inversions. The data are in agreement with earlier conclusions (STURTEVANT 1931) that homozygous inversions show about the same total amount of crossing over as do homozygous normals. They are also in agreement with the conclusions of BEADLE (1932) and of OFFERMAN and MULLER (1932) that the distribution of this crossing over is altered by relation to the spindle attachment, a given section giving less crossing over if it is near the attachment, more if it is near the free end of the chromosome.

b. Heterozygous inversions. The effects on crossing over are, as might be expected, different for sections within the inversion and those outside it, and are also dependent on the length and position of the inversion concerned.

Crossing over within the inversion is evidently decreased in heterozygotes for In sc-7 and In dl-49 (pp. 586–588), and is probably decreased in In ClB since so few doubles are recovered. The other inversions studied here, all of them longer than these, seem to have much less effect on crossing over within the inversion, though the data are scarcely adequate to permit the conclusion that there is no effect.

Crossing over in sections outside the inversion is regularly reduced. In sc-4 and In sc-8 do not leave any sections uninverted in which crossing over occurs in normal flies and can be measured in inversion heterozygotes. The same is true for the sections to the left of In sc-7 and In y-4 and for that to the right of In Df (bb). The remaining seven uninverted sections all show a reduction in crossing over, localized close to the inversion itself to the right of In sc-7, relatively slight to the right of In dl-49, and very marked in the other cases, namely, on both sides of In ClB, to the left of In dl-49 and of In Df (bb), and to the right of In y-4.

These data are in approximate agreement with expectations from the competitive pairing hypothesis of DOBZHANSKY (1931). A short inversion may be supposed to have its pairing more interfered with by the uninverted sections than does a long inversion which has shorter uninverted sections. Conversely, a long inversion may be supposed to interfere more seriously than a short one with the pairing of the uninverted sections.

One other relation is suggested here, as it is by the data on autosomal inversions (STURTEVANT 1931); namely that an inversion is more effective in suppressing crossing over in segments distal to itself than in proximal segments. This relation is difficult to analyze, and of the present series of inversions dl-49 seems to be the only one favorable for its study. What is needed is a comparative study of a larger series of more diverse types of inversions than we have used. This need is supplied in part by STONE and THOMAS (1935), who reach conclusions similar to the one just suggested.

THE EFFECTS OF THE Y CHROMOSOME ON CROSSING OVER

The most extensive series of data on the effects of a Y chromosome on crossing over is that of BRIDGES and OLBRYCHT (1926). DR. BRIDGES informs us that the XXY females there recorded gave, in addition to the published results, 246 exceptional offspring (2.5 per cent). Correcting the data (by adding twice the number of exceptions to the non-crossover class in the case of XXY females) gives the following comparisons:

	sc	*ec*	*cv*	*ct*	*v*	*g*	*f*	total	N
XX	6.7	8.8	8.2	14.4	11.3	12.3		54.5	11325
XXY	6.5	9.1	8.3	14.2	10.1	9.7		50.8	9461
XX/XXY	1.03	.97	.99	1.01	1.12	1.27		1.07	

Evidently the Y has no effect on crossing over in the region from *sc* to *v*, reduces *v-g*, and reduces *g-f* still more. This is in agreement with the results on duplications described by DOBZHANSKY (1934), since the Y is homologous only with the right end of the X, and reduces crossing over only in the portions of the X near this homologous section.

The results recorded in this paper for comparable XX and XXY females can also be interpreted in terms of the hypothesis of "competitive pairing" (DOBZHANSKY 1931). In Df(bb)/+ gives no effect of the Y, as might be expected, since Df(bb) presumably carries little or no material homologous to the Y. In the cases of sc-4/+ and y-4/+ there is an increase of double crossing over within the inversion, which may be looked upon as due to interference of the Y with pairing of the attachment ends of the X's, this in turn leading to less interference of these attachment ends with full pairing of the inverted segment. In the case of dl-49/+ the crossing over studied is that between the inversion and the spindle attachment; the data are in agreement with the analysis just given in that they indicate a decrease in crossing over due to the Y. The one remaining case in which we have comparable data is that of y-4/Df(bb), where the frequency of singles within the common inverted region seems to be unaffected by the presence of a Y, as would have been expected.

SECONDARY NON-DISJUNCTION

XXY females of all kinds give segregation of two chromosomes to one pole, one to the other (X-XY or XX-Y). The inversions affect the relative frequencies of these two types, and therefore a fuller understanding of the meiotic behavior of inversions should throw light on the mechanism of secondary non-disjunction.

If p be taken as the frequency of XX-Y segregation, then assuming random fertilization by X sperm and Y sperm and death of the XXX and YY classes, the frequency of recovered exceptions, q, will be $\frac{p}{2-p}$.

The earlier analyses of secondary non-disjunction (BRIDGES 1916, ANDERSON 1929, GERSHENSON 1935) have been based on the assumption that the maximum frequency of XX-Y segregation occurred when one X separated from Y and the other X went to either pole at random. This gives $p = .5$, $q = .333\%$. GERSHENSON himself obtained, from In ClB/+, $q = .353 \pm .0040$ in the female classes, a deviation about five times the probable error. Adding our data the value becomes $.366 \pm .0038$, a deviation of .033 or nearly 9 times the probable error. In the case of In dl-49/+ the female data of table 14 give $q = .456 \pm .0051$, a deviation of .123, 24 times the probable error.

There can be no doubt, then, that XX-Y segregation can occur with a frequency greater than 0.5. As a matter of fact the dl-49/+ value for q (.456) gives $p = .626$.

It becomes necessary, therefore, to search for a new interpretation of secondary exceptions. As pointed out by Bridges (1916), nearly all the exceptional females from +/+/Y mothers are non-crossovers, carrying the same two X's as their respective mothers. The same relation holds for In/+/Y exceptions, as shown by GERSHENSON (1935) and by our own data. In both types of experiments occasional exceptions are found with crossover chromosomes; but these are little, if any, more frequent than are such crossover exceptions from XX mothers; they may safely be disregarded in analyzing the effect of the Y on non-disjunction. Secondary exceptions, then, carry two unlike non-crossover chromatids. The failure of separation must take place at the first meiotic division, rather than the second, since the latter would give two *like* chromosomes. This is true provided Drosophila agrees with plants in having the first division reductional for spindle attachments. Indirect evidence as well as direct cytological evidence (KAUFMANN, 1935) indicates that this assumption is correct. It may be concluded also that secondary exceptions result from X-*tetrads* in which no crossing over occurred, for otherwise one would have to assume that the orientation of sister chromatids on the second meiotic spindle was not random. This is contrary to what is known in other cases; and even this assumption would not suffice to account for 3-strand or 4-strand doubles.

If secondary exceptions arise from non-crossover X tetrads, the next step is to determine the frequency of such tetrads in various kinds of females and to compare this with the frequency of non-disjunction in such females. This can be done best in the case of In dl-49. As shown above, XXY dl-49/+ females gave about 1 percent exchange to the left of the inversion, 12 percent in the inversion, and 20 percent to the right of the inversion. There are probably not over 3 percent multiple crossovers here, so 30 percent is not far from the true value for the crossover X-tetrads. (Three-strand multiples where one crossover is in the inversion and one is outside it should give rise to inviable eggs; the data presented above show that these are negligible in frequency.) Therefore, among the 70 percent non-crossover X tetrads, 62.6 or 90 percent give rise to non-disjunctional gametes. If we use the frequency of exceptional females actually observed in the same experiment in which crossing over was studied, we find that 66/79 or 83 percent of the non-crossover X tetrads gave XX-Y segregation. This does not take into account exchanges within the inversion. There can be little doubt that 90 percent is too low rather than too high a value. If one assumes that this same proportion holds in all cases the resulting deduced frequencies of complete non-crossover X tetrads (for example 9 percent for +/+/Y) seem not unreasonable. In any case, the proportion .667 suggested by BEADLE and STURTEVANT (1935) by analogy with the fourth chromosome is clearly incorrect for dl-49/+.

Table 14 shows that the frequency of secondary exceptions rises as the total frequency of crossing over decreases in the various inversion combinations. Changes in the reverse direction have not been recorded in *D. melanogaster*, but other species give more crossing over; that is, they have longer crossing over maps and presumably fewer non-crossover tetrads. The available data are shown in table 24. The map lengths given are probably too short, especially in *willistoni* and *pseudoobscura*, owing to fewness of available loci for study. Other species have been omitted because this element of uncertainty is even greater. It is clear that the table is in agreement with expectation.

TABLE 24

Comparison of species.

SPECIES	SECONDARY EXCEPTIONS		TOTAL MAP LENGTH OF X	
	%	AUTHORITY	UNITS	AUTHORITY
melanogaster	4.3	Bridges 1916	66	Bridges, unpubl.
simulans	2.9	Sturtevant 1929	70	Sturtevant 1929
willistoni	1.7	Lancefield and Metz 1921	84	Lancefield and Metz 1922
virilis	0.5	Kikkawa 1932	182	Kikkawa 1932
pseudoobscura	0	Schultz and Redfield, unpubl.	170	Lancefield 1922

The frequency of secondary exceptions thus shows strong negative correlation with the frequency of tetrad crossing over. Since the latter value is not greatly affected by the presence of a Y, whereas the former is, it may be concluded that the frequency of secondaries is dependent on the occurrence of non-crossover tetrads, rather than the reverse.

NORMAL DISJUNCTION OF X'S

In the case of dl-49/+ the data show about 14 percent crossing over (28 percent exchange) between the spindle attachment and the inversion. The attached-X data show about 12 percent exchange within the inversion. These results are from XXY females; in the cases of other inversions the presence of a Y has been shown to give a slight increase in crossing over within the inversion. There is a small percentage (about 1 percent) of exchange between the inversion and the free end. The indicated total frequency of exchange is thus 41 percent. There is a fairly large probable error attached to this value; but, since there are probably some double exchanges involved, it seems safe to conclude that at least half the tetrads undergo no exchange.

The data of table 14 show, from this combination, no matroclinous females in a total of 3238 daughters. It follows that exchange is not necessary for normal disjunction. This conclusion can be avoided only by supposing that undetectable exchanges occur between the known genes and the attachment end of the chromosome. This supposition has no evidence in its support and is made unlikely by the absence of matroclinous females from In Df(bb)/+ and their presence only in numbers similar to those given by +/+ in the cases of In sc-8/+ and In Df(sc-8)/+. These three cases all involve inversions that upset the homology well within the inert region, and might well be expected to interfere with crossing over in the region concerned.

The results here reported may, then, be taken as supporting the conclusion, which is probable on other grounds, that crossing over is not a necessary requirement for regular disjunction of the X chromosomes of *Drosophila melanogaster*.

POPULATION MECHANICS OF INVERSIONS

The scheme for inversion crossovers here developed should apply in all cases of inversions that do not include spindle attachments, since singles within such inversions should always give ties between first meiotic nuclei. The resulting selective eliminations of crossover chromatids may be expected in any case where three of the four products of meiosis are eliminated, the effective one being terminal (in terms of the orientation of the second division spindles). These conditions hold in the oögenesis of most animals and in the megasporogenesis of most seed-plants. In such forms as the Ascomycete Neurospora, where all the products of meiosis are potentially functional but are still arranged in a line, inversions of the type under discussion should lead to non-functioning of "inner" nuclei in much higher proportions than terminal ones. In plants the result will be the production of numerous inviable pollen grains, but no increase in egg inviability. There will therefore be no decrease in fertility, a circumstance that must prevent the rapid elimination of inversions through a reduced rate of reproduction. In animals the aberrant sperm will presumably be viable and functional, but will lead to the production of inviable zygotes and therefore to reduced fertility. In Drosophila this result is not brought about because of the absence of crossing over in the male.

A mechanism that increases the number of gametes carrying a single complete haploid set of chromosomes exists also in the case of heterozygotes for reciprocal translocations, where there is a higher frequency of "regular" than of "irregular" gametes in most cases. Here, however, there is no marked sexual difference, and the frequency of irregulars is high enough to produce an appreciable decrease in fertility in most (probably in all) cases. These relations are probably responsible for the observation that, within a given species of Drosophila, wild populations carry inversions far more frequently than translocations.

Inversions that include the spindle attachment cannot produce a chromatid tie, and will therefore decrease fertility if single exchanges occur within them. This is probably the explanation of the fact that no such inversions have been found in wild populations of Drosophila, though they do occur as a result of X-ray treatment.

SUMMARY

1. Seven inversions are discussed. Their nature is illustrated in figure 1.

2. The results obtained from females heterozygous for two inversions are described. The properties of the chromosomes produced by single crossing over within the common inverted sections are summarized in table 11.

3. The frequencies of matroclinous females and patroclinous males from the combinations studied are shown in table 14.

4. Females carrying attached X's, in one of which there is an inversion, give rise to closed X's by single crossing over within the inversion.

5. Egg counts show that the mortality from inversion heterozygotes can all be accounted for as due to the fertilization of no-X eggs by Y sperm. This is very much less than the indicated frequency of single crossover chromatids.

6. Since single crossovers are produced but are not recovered, they must be eliminated from the egg at meiosis, leaving a non-crossover chromatid in the reduced egg.

7. A scheme for such oriented divisions is shown in figures 6 and 7. This is based on cytological observations on plants and on the observed geometrical relations of the meiotic divisions in the Drosophila egg.

8. According to this scheme, crossover chromatids with two spindle attachments form ties between two nuclei at the first meiotic division, resulting in the tied chromatid failing to pass to either terminal pole; or at the second division, resulting in death of the egg when the egg nucleus is concerned.

9. This scheme results in several numerical predictions, which are borne out by the data:
   (a) matroclinous females from XX mothers are not increased in frequency by inversions.
   (b) patroclinous males are to recovered double crossover males as 2:3.
   (c) egg mortality is practically equal to the frequency of patroclinous males.

10. Inversions, and also the presence of a Y chromosome, decrease crossing over in accordance with the hypothesis of competitive pairing.

11. Females (XX) heterozygous for inversions may give many no-exchange tetrads; these segregate normally, with the production of no significant number of XX gametes.

12. In XXY females that are dl-49/+, 90 percent or more of the eggs in which no exchange occurs give XX-Y segregation. Similar frequencies are probable in all cases studied.

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CHROMOSOME STRUCTURE X. AN X-RAY EXPERIMENT†

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INTRODUCTION

IT WAS recently found (NEBEL and RUTTLE, in press) that *Tradescantia reflexa* Raf. shows four threads[1] per chromosome during all somatic as well as during all premeiotic stages.

Multiplication of threads occurs at metaphase during somatic division (fig. 1). In meiosis of *T. reflexa* the four threads multiply at early interkinesis and do not do so again until the metaphase of the first pollen division (fig. 2). The leptotene thread is four-partite. This corresponds to the four-partite structure of chromosomes in mitotic prophases. The chromosome at interkinesis contains eight threads per univalent while at the quartet stage each chromosome again contains four threads. The orientation of these threads disregarding the coils is equidistant for only a short time during telophase. During the remainder of the cycle the chromosome consists essentially of two well-defined chromatids which can only with special precautions be resolved into longitudinal half-chromatids. This orientation is represented by figures 1 and 2.

To demonstrate the quadripartite nature of the presynaptic or leptotene chromosome X-rays were used in order to determine whether such treatment might bring about lesions affecting only one of the constituent threads of a chromosome. If it is possible to influence the discrete threads individually, the cytological study of their subsequent behavior may well complete the demonstration of the quadripartite nature of the presynaptic chromosome. These lesions, while related to the peculiar spatial and functional order of the four threads in a chromosome, seemed to vary in type with variation in dosage, as will be explained below.

LITERATURE AND PRELIMINARY DISCUSSION

X-rays have been used to determine the number of chromonemata in chromosomes by several investigators. According to the majority the chromosome is bipartite during most stages of the cycle. The present find-

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[1] The term "thread" will be used as a morphological term for such ultimate filamentous constituents of the chromosome as can be resolved optically. The term *chromonema* is avoided because it has recently been confused with chromatid. *Genonema* carries theoretical implications and should be reserved for the time when the physical structure of the gene will have been more fully resolved.

ing that the chromosome is four-partite may perhaps be harmonized with that of other investigators under the following premises:

(a) Tradescantia is favorable material with large chromosomes; other material such as Drosophila may have chromosomes which cannot be divided into four parts by either optical or genetical means.

(b) Most workers raying liliaceous plants have not recognized four-partite chromosomes as a normal phenomenon and have therefore not been looking for results in their experiments which might indicate their presence.

(c) Half-chromatids are broken only under certain conditions of dosage and at certain stages. Whether they can be broken in mitosis as well as in meiosis is not certain.

LEWITZKY and ARARATIAN (1931), radiating somatic cells of Secale, found after 2 days a large number of fragments some of which were very small. These small fragments they believe may have come from parts of the chromosome which are naturally attenuated (the kinetochore and its neighborhood). According to the present view they may come from fragmentation of one of the four constituent threads of the chromosome. STONE (1933) and MATHER and STONE (1933), raying somatic tissue of liliaceous plants, observed only breaks of entire chromosomes. MATHER (1934) rayed cymes of Tradescantia and observed the resulting chromosome changes from 3 to 24 days after the treatment. Although MATHER did not use methods which would show the threads plainly, his figure 18, showing first pollen grain divisions nine days after radiation, indicates that the chromosome is four-partite before meiosis. SAX and EDMONDS (1933) give a time schedule for the development of the pollen grains in Tradescantia, according to which the material which MATHER shows in his figure 18 was apparently rayed before synapsis. Small fragments of about half the diameter of a chromatid are also present in figure 11 of HUSKINS and HUNTER (1935), who radiated microspores of Trillium after the second reduction division and observed the first division in the pollen grain. These results indicate that breaks in half-chromatids may be observed in radiation of nuclei of the somatic type. MARSHAK (1935) concludes from his work on Gasteria that the chromosome previous to synapsis is "at least two-partite."

According to earlier findings (NEBEL and RUTTLE in press), the chromosome in Tradescantia is visibly four-partite from the last premeiotic metaphase until early interkinesis (fig. 2). Each constituent thread then multiplies, the eight resulting threads forming sister chromosomes of the second meiotic division. The next multiplication of threads occurs at metaphase of the first division of the pollen grain. Any normal chromosome in a metaphase plate of the first division in the pollen grain is thus the direct descendant of one chromatid of pre-meiosis. Any surviving fragments of half-chromatids produced at pre-meiosis would, if they divided normally, appear as longitudinal half-chromosome (chromatid) fragments in the early metaphase of the first division of the pollen grain. When the original fragments survive but are too small to be capable of division, they will show up in the pollen grain division as minute bodies of about one-fourth the diameter of a normal chromosome.

The observations of LEVITZKY and ARARATIAN (1931) on somatic cells of rye can be interpreted according to the present view point. The small fragments shown in their figure 2 plate 1 from divisions fixed two days after radiation may be fragments of half-chromatids. The authors vainly seek an explanation of these very small fragments. MATHER (1934) also shows small fragments in his figure 18. These, as well as the large chromosome pieces which consist of only one chromatid of prometaphase of the pollen grain division, are interpreted to be the direct descendants of breaks of half-chromatids, which occurred during presynapsis. HUSKINS and HUNTER (1935) likewise probably obtain breaks in half-chromatids of Trillium, indicating that the chromosomes of this plant are four-partite at all stages. MARSHAK (1935) considers the chromosome at least two-partite before synapsis. MARSHAK was working with relatively low dosages of X-rays which according to the present results give only a very low frequency of half-chromatid breaks or none at all. For this reason MARSHAK's findings are considered not to contradict the present work. Thus it may be said that the data of the literature while not directly so interpreted by the respective investigators may be in actual agreement with the fact that the chromosomes of many monocots are four-partite at all stages.

This conclusion apparently does not hold for animal material as far as it has been carefully investigated. PATTERSON (1933 and 1935) and MOORE (1935) have shown from genetical evidence that the interphase chromosome of Drosophila is a bipartite structure. Radiation of mature sperm gave complete mutations as well as mosaics. The latter indicate that there are two gene threads per chromosome in the resting stage. Why both threads may be affected identically by radiation will be discussed later. WHITE (1935) rayed spermatogonia of Locusta and observed various types of lesions during the first mitosis, which occurred after treatment. When metaphases were observed 32 hours after radiation WHITE found chromatid breaks, indicating that the chromosome was split in two at the time of radiation. WHITE's data thus indicate that orthopteran chromosomes are physiologically double at all stages of the mitotic cycle.

MATERIALS AND METHODS

Cymes (flowering heads) of plants of *T. reflexa* were radiated with 50, 200, 500 and 1000 *r* units respectively. A Coolidge tube running at 180 k.v.p. and 3 milliamperes furnished the radiation. All buds containing stages later than diakinesis were removed previous to exposure. It is assumed that the majority of the sporocytes subjected to radiation contained presynaptic nuclei for the following reason. In *T. reflexa* reduction division roughly follows a daily cycle, and successively younger buds in a vigorous half-cyme show stages separated by a definite interval. From leptotene to first metaphase requires approximately 24 hours. If all buds later than diakinesis are removed in a given cyme, it is safe to say that all remaining buds are in presynapsis at that time. In less vigorous cymes such as were used in the present experiment, successively younger buds show even greater time intervals.

Smear preparations were made in the following way: pretreated in ½ normal Ringer 30 seconds at pH 7.5; fixed in 3:1 alcohol acetic acid; stained with carmine alum lake in 45 percent acetic acid; dehydrated for two minutes each in alcohol acetic acid 2:1, 4:1, 10:1, absolute alcohol, clove oil; mounted in diaphane. This method yields slides which will keep for a few months. The use of clove oil was suggested by M. L. RUTTLE (unpublished). Had it not been for this method the half-chromatid breaks described in this paper would undoubtedly have escaped observation.

OBSERVATIONS

The microscopic evidence of half-chromatid and chromatid lesions of presynapsis is shown in figure 3, A–D. These chromosomes are from cells radiated during presynapsis; A, B and C are from second anaphase, D is from prometaphase of the first division in the pollen grain. The chromosome of second anaphase contains four threads, two in each chromatid. In figure 3, A and B there are visible asymmetries between sister chromatids. Since each chromatid of the second anaphase is the equivalent of a half-chromatid of the cycle preceding first metaphase, lesions as shown in figure 3, A and B are termed half-chromatid lesions of presynapsis. Figure 3C shows an entire chromosome of second anaphase, which forms a slight angle at the insertion region. On the vertical arm there are two achromatic spots on sister chromatids which are due to a lesion of a presynaptic chromatid. Figure 3D is from prometaphase of the first division in the pollen grain. The chromosome is obviously split at this stage into the two prospective chromosomes of anaphase and each half contains two threads which are about to become four. The asymmetric pieces towards 10 and 2 o'clock are prospective anaphase chromosomes which have no sister halves. The asymmetry of prometaphase chromosomes of the first division in the pollen grain corresponds to lesions which affected half-chromatids of presynapsis. With reference to figure 3, A and B it may be said that they are presented as examples of a phenomenon which appeared only under the specific conditions of radiation of 200 *r* units and above. The origin of each single case might be questioned but the occurrence of this type of lesion exclusively under the condition of the experiment renders the individual case significant.

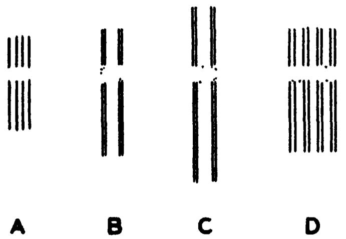

FIGURE 1. Diagram of Mitosis. A, telophase with threads equidistant B, interphase C, prophase. D, metaphase. Multiplication of threads becomes visible during metaphase as soon as the prospective chromosomes of anaphase are ready to separate. Coiling has been disregarded; also the threads are not actually arranged in a single plane.

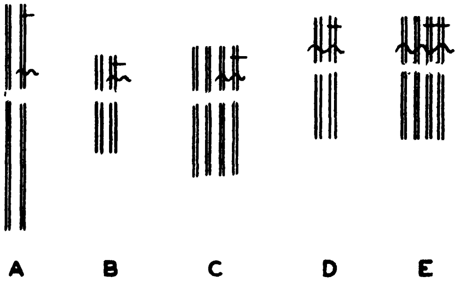

FIGURE 2.—Diagram of number of threads in a single chromosome in meiosis and metaphase of first division in the pollen grain. A, leptotene. B, first metaphase C, interkinesis. At this stage multiplication of threads becomes visible; each chromosome contains eight threads which separate at the second division. D, quartet stage (only one of the two chromosomes which separate in the second divi ion is shown). E, metaphase in first division of the pollen grain. Another multiplication occurs here. "Lesions" have been entered by cross bars, the straight horizontal bar illustrates the history of a lesion affecting a half chromatid of presynapsis. The wavy cross bar illustrates a lesion affecting one chromatid of presynapsis. A half-chromatid lesion of presynapsis becomes a chromatid lesion at second anaphase and a chromosome lesion after the metaphase of the first division in the pollen grain. A chromatid lesion of presynapsis becomes a chromosome lesion at second division etc.

Table I is a summary of the observations. The effect of radiation was observed primarily during first and second reduction division. Turning to figure 2, it can be seen that a lesion affecting one chromatid at leptotene (fig. 2A) would be observed as such during first division, (fig. 2B) but would during late metaphase and anaphase of the second division appear as a lesion affecting a chromosome (fig. 2D). At the division in the pollen grain this same lesion would affect both halves of the chromosome at prometaphase (fig. 2E).

Table 1

Effect of X-rays on presynaptic chromosomes observed during first and second anaphase

Dosage r units	Meiotic division	Hours after treatment	Total lesions	Counts of lesions affecting: Both homologues	One chromosome	One chromatid	1 half-chromatid	Percentage of lesions affecting: Both homologues	One chromosome	One chromatid	1 half-chromatid
1000	2nd	60.5	81	2	18	51	10	2.5	22	63	12.3
500	1st	53	46		2	42	2		4.4	91.2	4.4
	2nd	22	183		5	157	21		2.7	85.7	11.6
200	1st	26	35		2	32	1		5.7	91.4	2.9
	2nd	26	120		9	109	2		7.5	90.8	1.7
50	1st	29	55			55				100	
	2nd	29	32		1	32			3	97	

A lesion affecting one half-chromatid at leptotene becomes a lesion of half a chromosome in second division and of a whole chromosome in the anaphase of the division in the pollen grain. In table 1 observations made

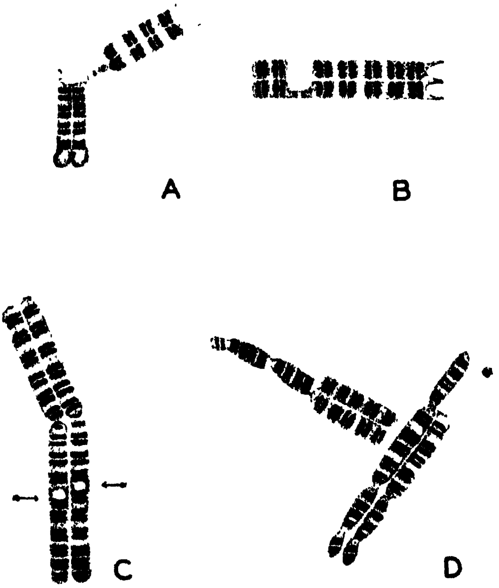

Figure 3.—Material radiated during presynapsis. A, B and C are from second anaphase, A, showing one chromatid asymmetrically attached to the end of another chromosome, B, showing one chromatid partly deleted. Half-chromosome (chromatid) lesions of second anaphase are the result of half-chromatid lesions of presynapsis. C illustrates two achromatic spots at the same level of a chromosome at second anaphase. These are the results of a lesion affecting one chromatid of presynapsis. D is from prometaphase of the first division in the pollen grain. The asymmetric pieces towards 10 and 2 o'clock are prospective anaphasic elements. Their sister halves have become detached. This corresponds to half-chromatid lesions of presynapsis. Camera lucida ×2400. (Drawn by M. L. Ruttle.)

on first and second divisions are reduced to the condition of the chromosome at first metaphase, which in Tradescantia is identical with the condition in leptotene or any other presynaptic stage after the last archesporial division. Thus if at second anaphase a lesion is observed which at that stage involves a single chromatid, this is entered in table 1 as a lesion having affected one half-chromatid.

The term lesion is used to include any type of cytological abnormality, such as achromatic spots, breaks or translocations, here again using the last term to describe the transfer of chromosomes or parts of chromosomes to previously unrelated parts of other chromosomes.

A lesion which affected a half-chromatid observed in second division could not be distinguished from a lesion affecting three half-chromatids. Breaks affecting three half-chromatids at first division were, however, not observed. Achromatic spots affecting a half-chromatid only were not observed. Achromatic spots seemed always to affect both halves of a chromatid (fig. 3c) and appeared in second division side by side as a clearly defined pair of spots or as narrow achromatic band across the width of a chromosome of second anaphase.

The data in table 1 seem to indicate that the effect of radiation changes with increasing dosage. At low dosages the effect is confined almost completely to lesions of chromatids. At higher dosages there is an increasing number of other lesions. Single half-chromatid lesions increase and lesions involving more than one chromatid at a given level become more numerous.

Since higher dosages of radiation merely represent longer exposures to the same type of radiation it is hard to understand why at the low dosage the types of lesions were narrowed to nearly a single class.

To explain the results it is suggested that under longer exposures the reaction of the chromatic elements is no longer a simple one. Under prolonged exposure heat may be generated in the cell, sensitive processes of metabolism may be invalidated sufficiently to change the reaction of chromatin toward radiation. In a general sense prolonged radiation causes a change in the environment of the chromosome threads, which in turn changes the type of reaction of the chromosomes toward radiation. Under low dosage, with no change in the environment, the unit of reaction within the chromosome is the chromatid. This is in keeping with the fact that crossing over is a reaction between chromatids, not between half-chromatids. It is also in keeping with the observation that half-chromatids are very closely united, perhaps by a common matrix.[2] It is assumed that sister half-chromatids form a physiological unit, which under moderate radiation reacts as a unit. Under increased radiation, due to a change in the environment the equilibrium between half-chromatids may become upset, and the normal physiological and spatial distance between related chromatids and chromosomes may also become disturbed. The effect of heavy radiation is similar to that of heat which causes clumping and sticking of chromatic elements which normally form no contacts.

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[2] I have suggested earlier (NEBEL 1932) that each chromonema is surrounded by its own individual matrix. In a general sense this is still maintained. If the diameter of the gene string (HASKINS 1935) is a fraction of that of a visible chromosome thread, this visible surrounding of

The present evidence is purely cytological but chromatic changes may entail genetic changes. The finding of STUBBE (1935) that the genom of *Epilobium hirsutum* is more sensitive to radiation when carried in *E. luteum* plasma than when carried in its own plasma may be quoted as an analogy for the instability of the genom under changes of environment.

The existence of half-chromatid breaks is not taken as proof but merely as a confirmation of the morphological observation that meiotic chromatids are formed and split in the last premeiotic metaphase in Tradescantia. It would be possible to explain the data differently but any alternative explanation leads into additional difficulties.

MARSHAK (1935) found a linear progression for lesions with dosage. The present material confirms MARSHAK'S finding. A special study of this phase was not possible but when a graph was drawn from the number of lesions observed under various dosages using only those cells in which all chromosomes could be seen, this graph showed 2 to 3 lesions per cell with 50 *r* units, 4 to 5 with 200, 8 to 10 with 500 and 10 to 18 with 1000 *r* units respectively. The graph is not given here because the numbers of perfect cells available for counting was low. Lesions involving a chromatid were counted as one, lesions involving a half-chromatid likewise. Lesions involving more than one chromatid were counted as one only where sister chromatids clearly appeared involved at the same level.

With higher dosages counts of lesions will appear low, for the reason that if a fragment becomes a micronucleus it will hide additional lesions which it may contain. Counts of lesions will appear high for the following reason. It is assumed that a single hit may at higher dosage spread "horizontally" —at right angles to the long axis of the chromosome. The first division separates homologous chromatids and the second division separates sister

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the gene string may be called a matrix. Other cytologists (KUWADA and NAKAMURA 1934) have postulated several matrices within the chromosome to account for the different coils which may be formed at one time within the chromosome. It may be well to maintain the term matrix and think of various matrices one inside the other, but these terms are merely descriptive images of what actually is effected by various forces of mutual attraction between adjacent threads. In the present case we must postulate one matrix to keep the four threads of a chromosome together. The second matrices hold chromatids at the correct distance and surround half-chromatids. The third matrices surround half-chromatids in Tradescantia. All three matrices are to our knowledge identical in substance.

chromatids. This separation makes it hard to detect lesions in corresponding regions of sister and homologous chromatids. The effect of what may have been a single hit will thus be considered multiple.

These two phenomena may cancel one another. With this in mind the obtained linearity of the curve of number of lesions per cell plotted against dosage suggests that under higher dosages the effect of a single hit may occasionally spread at right angles to the longitudinal axis of a chromosome, and also may, in contrast to the effect of low dosages, remain confined to a single half-chromatid. A few observations were made on the first somatic division in the pollen grain from cymes rayed with 500 *r* units. The divisions were observed 14 days after radiation. This time interval indicates that at the time of radiation the material was in presynapsis. Several divisions showed an abundance of fragments involving only one chromatid of prometaphase. From diagrams A to E of figure 2 it may be seen that such breaks would be expected from the lesion of half-chromatids of presynapsis.

The occurrence of half-chromatid breaks as observed at first and second division was followed thru successive days and could be traced for about a week after treatment. After this time half-chromatid breaks were no longer observed. This one would expect if the maximum time limit between reduction division and the last preceding archesporial division for any one cell were around 170 hours. This corresponds to the time schedule given by SAX and EDMONDS (1933).

SUMMARY

Radiation of cymes of *Tradescantia reflexa* Raf. was applied to presynaptic stages of microsporocytes. The effect of the radiation was observed during first and second reduction divisions. With low dosage the experiment indicates that the chromosome is split in *two* previous to synapsis. With higher dosage the experiment suggests that occasionally the chromosome is split into *four* previous to synapsis.

This is interpreted as follows: Each chromosome is composed of two split chromatids (or four half-chromatids) at all stages of presynapsis. In normal material the two half chromatids that constitute a given chromatid lie very close to one another and do not react individually. (The process of crossing over in *Tradescantia* is a reaction between chromatids in which every two half-chromatids behave as a physiological and mechanical unit.)

Under low dosage of X-rays half-chromatids are physiologically unable to show separate reactions.

With higher dosage of radiation (200 *r* units and more) the spatial and physiological order of the threads within a chromosome is upset. Chromatic reactions may be recorded which affect one of four (or three of four)

threads differentially. It is also indicated that with higher dosage chromatic reactions at a certain level of the chromosome may spread, involving sister and even homologous loci.

The differential effect of higher dosage is thus attributed to action upon the immediate environment of the chromatin, perhaps to heat or to a general interference with the normal metabolism of the nucleus which in turn changes the type of reaction of the chromatin.

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THE EFFECT OF AUTOSOMAL INVERSIONS ON CROSSING OVER IN THE X-CHROMOSOME OF DROSOPHILA MELANOGASTER

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INTRODUCTION

THE first evidence of a crossover suppressor was found in 1913 by STURTEVANT. In 1921 STURTEVANT found the first definite evidence of an inversion when he showed that several third chromosome genes in *Drosophila melanogaster* were arranged in an inverted order with respect to similar genes in the third chromosome of *D. simulans*. At this time STURTEVANT predicted that a heterozygous inversion would suppress crossing over and suggested that the Nova Scotia stock (STURTEVANT 1917 and 1919) had an inversion. It was not until 1926, however, that it was actually demonstrated that in at least one case crossover suppression was due to an inversion (STURTEVANT 1926).

The sole effect on crossing over ascribed to inversions until recently is that the number of recovered crossovers is reduced in the chromosome in which the inversion is located. But as long ago as 1919, STURTEVANT suspected that crossover suppressors (inversions) might have an interchromosomal effect. He reported that a high value for crossing over between black and purple was due, in part at least, to a dominant gene (really an inversion) in the third chromosome, which when heterozygous reduced crossing over in this chromosome.

WARD (1923) tested the effect of the curly inversion on crossing over in the first and third chromosomes; her observations on crossing over were of no value because of the small number of flies used, but she did notice an increase in multiple crossovers. PAYNE (1924) tested the effect of the Payne inversion on crossing over in the first and second chromosomes. While he counted large numbers of flies, the chromosomes were poorly marked (*$w^e m$* on the first chromosome and *b pr c sp* on the second) and he ran no controls; he therefore noticed no interchromosomal effect on crossing over. PAYNE made no statement in regard to the number of multiple crossovers recovered.

The problem rested here until 1932, when SCHULTZ and REDFIELD (MORGAN, BRIDGES and SCHULTZ 1932) stated that inversions in the first and second chromosomes increased crossing over in the third chromosome. In the following year they published a short account (MORGAN, BRIDGES and SCHULTZ 1933) in which they stated that inversions in the first and third chromosomes increased crossing over in the second. The increased crossing over in both cases was due to an increase in multiple crossing over.

The experiments reported in this paper are concerned with the effects of inversions in the second and third chromosomes on crossing over in the first chromosome.

MATERIALS AND TECHNIQUE

The autosomal inversions used in the experiments were the Payne inversions (C3LP *Dfd* C3RP *ca*) in the third chromosome and the Cy inversions (Cy C2L.C2R$_{Cy}$) in the second chromosome. The Payne inversions (PAYNE 1924) reduce crossing over almost completely throughout the length of the third chromosome. The Curly inversions (WARD 1923) gave no crossover flies in a total of 2487 from a cross of heterozygous Cy/2ple females by 2ple/2ple males. Crossing over in the first chromosome was detected by means of the alternated X^7 stock (*y cv v f/ec ct*^{6} *g*^{2}). This is identical with the alternated X-ple stock (BRIDGES and OLBRYCHT 1926) except for the substitution of *y* for *sc*.

The crosses were as follows:

1. +/+; +/+; *y cv v f/ec ct*^{6} *g*^{2} ♀ ×Oregon-R ♂ (controls)
2. +/+; +/Cy; ″ / ″ ♀× ″ ♂
3. +/Payne; +/+; ″ / ″ ♀× ″ ♂
4. +/Payne; +/Cy; ″ / ″ ♀× ″ ♂

Because of a significant deviation of the crossover value in region 1 of the controls from the standard value (to be discussed below) it is important that the relation of the chromosomes of the test stocks to those of the controls be explained. The derivation of the stocks was such that one set of autosomes and one X chromosome in each stock came from the original *ec ct*^{6} *g*^{2} stock. The second X chromosome in each case came from the original *y cv v f* stock. The origin of the remaining chromosomes was as follows: in the controls they all came from the *y cv v f* stock. In the +/+; +/Cy; *y cv v f/ec ct*^{6} *g*^{2} stock the remaining third chromosome had an equal chance of coming from either the *y cv v f* or the Curly stock; the same holds true for the fourth chromosome. In the +/Payne; +/+; *y cv v f/ec ct*^{6} *g*^{2} stock the remaining second and fourth chromosomes had an equal chance of coming from either the Payne or the *y cv v f* stock. Since twenty cultures with from two to three females in each were counted for each cross, it is likely that the origins of these chromosomes were very nearly equally divided between their possible sources. In the +/Payne; +/Cy; *y cv v f/ec ct*^{6} *g*^{2} stock only the origin of the fourth chromosome is in doubt. It had an equal chance of coming from either the Payne, the Curly or the *y cv v f* stock.

In all the crosses the male offspring only were classified for each of the seven first chromosome characters involved. The crosses were run at 25 ± 1°C on the usual cornmeal-agar-molasses food medium.

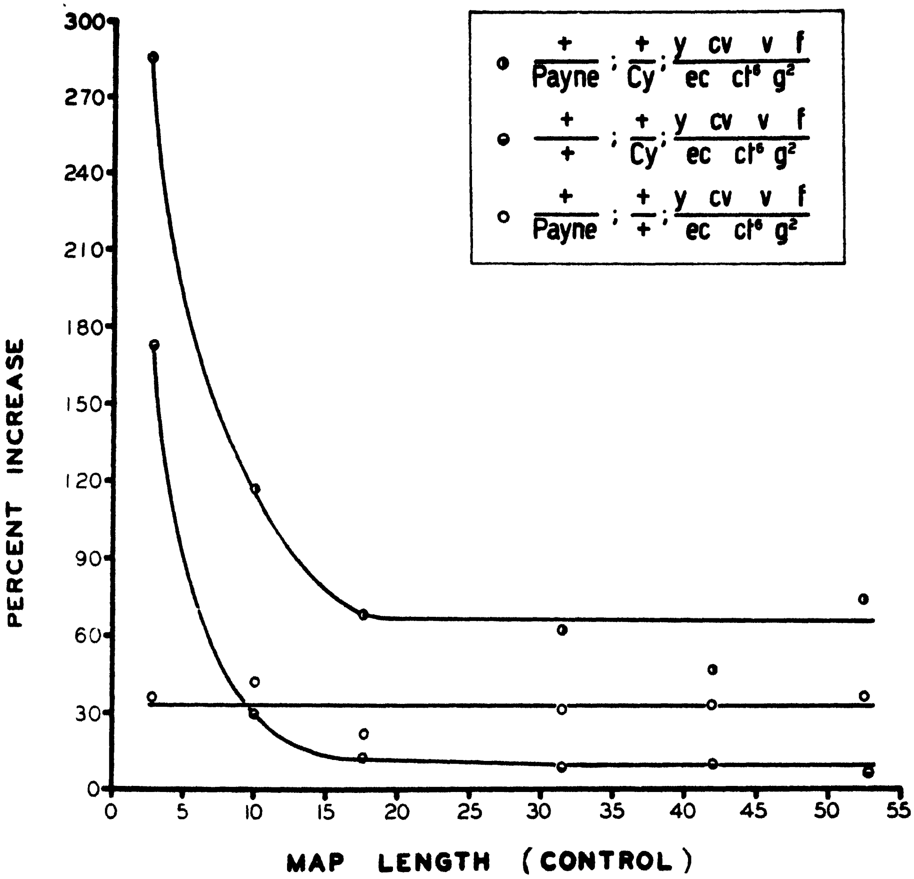

FIGURE 1 —Plot of percent increase in crossing over in the test crosses over that of the controls against the control map length. (Data from table 1.)

THE DATA

The crossover values in the controls for all regions except region 1 compare favorably with the standard map distances (table 1). Region 1, however, gives a value which is but half that of the standard value. Since a large number of flies was counted (3937) and classification was certain, this difference must be considered significant. The construction of the stocks used in the test crosses makes it unlikely that this low value is due to any autosomal modifiers. Since the source of the X chromosomes is constant throughout the experiment it is almost certain that the cause of this low value in region 1 of the controls had no effect on the relative values of the results, although the possibility remains that there might have been some effect on the absolute values.

Table 1 shows the crossover values for each of the four different crosses as well as a comparison of the crossover values of the test crosses with those of the controls. These values are calculated from the raw data shown in table 5. There is an increase in crossing over in each of the six regions of the test crosses as compared with the controls. This increase is greatest in the cross involving both sets of inversions, next greatest in the cross with the Payne inversions and least in the cross with the Curly inversions (table 1, fig. 1). This is due to a large increase in multiple crossovers which is again greatest in the cross involving both sets of inversions, and least in the cross involving the Curly inversions.

TABLE 1

Comparison of crossover values.

REGION	1		2		3		4		5		6		MAP LENGTH		TOTAL NUMBER OF FLIES
	% CROSSOVER	% INCREASE	% CROSSOVER	% INCREASE	% CROSSOVER	% INCREASE	% CROSSOVER	% INCREASE	% CROSSOVER	% INCREASE	% CROSSOVER	% INCREASE	MAP LENGTH	% INCREASE	
$\frac{+;+;y\ cv\ v\ f}{+;+;ec\ ct^6\ g^2}$	2.7	—	7.4	—	7.7	—	13.6	—	10.7	—	10.7	—	52.8	—	3937
$\frac{+;+;y\ cv\ v\ f}{+;Cy;ec\ ct^6\ g^2}$	7.4	171.1	9.8	32.4	8.7	13.0	14.8	8.8	11.9	11.2	11.5	7.5	64.1	21.4	2733
$\frac{+,+;y\ cv\ v\ f}{Payne,+;ec\ ct^6\ g^2}$	3.7	37.0	10.6	43.2	9.2	19.5	17.8	30.9	14.3	33.6	14.8	38.3	70.4	33.3	3612
$\frac{+;+;y\ cv\ v\ f}{Payne;Cy;ec\ ct^6\ g^2}$	10.4	285.2	16.1	117.6	12.9	67.5	22.2	63.2	16.0	49.5	19.4	75.4	97.0	83.7	1554

The Curly inversions have their greatest effect on the left end of the X chromosome, causing an increase in crossing over of 171.1 percent between *y* and *ec* (region 1, table 1). The effect drops rapidly as the right end of the chromosome is approached so that between *cv* and ct^6 (region 3, table 1) the increase is only 13.0 percent. From this point on the effect on crossing over is approximately constant (table 1, fig. 1).

The Payne inversions, unlike the Curly inversions, have an approximately uniform effect on crossing over throughout the portion of the X chromosome under observation in this experiment (table 1 and fig. 1). This effect is to cause an increase of approximately 33 percent in each of the six regions. According to SCHULTZ and REDFIELD (MORGAN, BRIDGES and SCHULTZ, 1933), the Payne inversions did not have a uniform effect throughout the length of the second chromosome but affected crossing over in this chromosome in much the same manner as does triploidy. Why the effect of the Payne inversions on one chromosome is different from their effect on another cannot be explained at present.

The Curly and Payne inversions when combined affect crossing over in the first chromosome in somewhat the same manner as do the Curly inversions alone (table 1 and fig. 1). Here again, the increase is greatest in region 1 (285.2 percent) and decreases rapidly to 65.7 percent in region 3 and remains practically constant thereafter (table 1 and fig. 1). The effect of the combined inversions is greater in every region than is the effect of the inversions taken singly. Furthermore, the effect of the combined inversions exceeds in every region the sum of the effects of each of the two pairs of inversions used separatelv (table 1).

The increase in crossing over noted in the crosses involving the inversions singly or together is due to an increase in multiple crossover strands and a decrease in non-crossover strands (tables 2, 3 and 4). In the cross

TABLE 2

Classification of strands into crossover classes

	CONTROLS $\frac{+}{+},\frac{+}{+},\frac{y\;cv\;v\;f}{ec\;ct^6g^2}$		$\frac{+}{+};\frac{+}{Cy};\frac{y\;cv\;v\;f}{ec\;ct^1\;g^2}$		$\frac{+}{Payne};\frac{+}{+};\frac{y\;cv\;v\;f}{ec\;ct^6\;g^2}$		$\frac{+}{Payne},\frac{+}{Cy};\frac{y\;cv\;v\;f}{ec\;ct^6\;g^2}$	
	NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT	NUMBER	PERCENT
non-crossovers	2052	52.1	1213	44.4	1532	42.4	470	30.2
singles	1697	43.1	1292	47.3	1632	45.2	702	5.2
doubles	181	4.6	224	8.2	430	11.9	344	22.1
triples	7	0.2	4	0.1	16	0.4	37	2.4
quadruples	—	—	—	—	2	0.1	—	—
quintuples	—	—	—	—	—	—	1	0.1
totals	3937		2733		3612		1554	

TABLE 3

Showing comparison of tetrads having a crossover in a given region with those having a crossover in the given region and in one other region. All values are in percent.

REGIONS	1		2		3		4		5		6	
	SINGLES	DOUBLES	SINGLES	DOUBLES	SINGLES	DOUBLES	SINGLES	DOUBLES	SINGLES	DOUBLES	SINGLES	DOUBLES
$\frac{+}{+};\frac{+}{+};\frac{y\;cv\;v\;f}{ec\;ct^6\;g^2}$	2.8	2.3	9.9	4.4	9.8	5.0	19.9	2.8	16.1	5.0	10.4	6.5
$\frac{+}{+};\frac{+}{Cy};\frac{y\;cv\;v\;f}{ec\;ct^6\;g^2}$	6.7	7.7	13.8	8.7	9.2	7.9	17.9	11.1	11.3	11.8	7.3	15.1
$\frac{+}{Payne};\frac{+}{+};\frac{y\;cv\;v\;f}{ec\;ct^6\;g^2}$	2.3	4.6	7.0	11.9	6.8	11.6	14.0	20.1	11.0	15.3	4.4	23.0
$\frac{+}{Payne};\frac{+}{Cy};\frac{y\;cv\;v\;f}{ec\;ct^6\;g^2}$	1.4	11.4	−1.3	26.0	5.9	13.4	6.6	21.0	2.8	21.3	1.0	27.6

with the Curly inversions the double crossover strands are increased by 78 percent (table 2). The non-crossover strands are decreased from 52.1 percent to 44.4 percent, a reduction of 14.8 percent. There is an apparent reduction in the number of triple crossovers but the numbers involved are small and therefore the reduction is probably not significant. The increase in single crossover strands is probably significant. ($D/\sigma_d = 3.5$.) The Payne inversions cause an increase in double crossover strands of 154 percent and a decrease of 18.4 percent in the non-crossover strands. The single crossover strands do not change (43.1 percent in the controls and 45.2 percent in the Payne cross). Here again the numbers of three point crossover strands are too small to give decisive evidence as to the effect on triple crossovers. The combined inversions have a great effect on both the mul-

TABLE 4

Distribution of crossovers in tetrads All values are in percent. Symbols are explained in the text. (The 5 point crossover is not included in the calculations of $T_{\cdot i}$, $T_{\cdot ij}$, etc.; the values of the T_{ijk} tetrads are omitted.)

CLASS	$\frac{+}{+};\frac{+}{+};\frac{y\ cv\ v\ f}{ec\ ct^6\ g^2}$	$\frac{+}{+},\frac{+}{Cy},\frac{y\ cv\ v\ f}{ec\ ct^6\ g^2}$	$\frac{+}{Payne};\frac{+}{+};\frac{y\ cv\ v\ f}{ec\ ct^6\ g^2}$	$\frac{+}{Payne};\frac{+}{Cy},\frac{y\ cv\ v\ f}{ec\ ct^6\ g^2}$
T_0	13.4	5.2	8.8	4.7
$T_{\cdot i}$	68.9	62.4	44.4	16.4
$T_{\cdot ij}$	16.2	31.6	45.2	59.6
$T_{\cdot ijk}$	1.4	0.8	0.0	19.2
$T_{\cdot ijkl}$	—	—	1.6	—
REGION		BY REGIONS		
1	2.8	6.7	2.3	1.4
2	9.9	13.8	7.0	−1.3
3	9.8	9.2	6.8	5.9
4	19.9	17.9	14.0	6.6
5	16.1	11.3	11.0	2.8
6	10.4	7.3	4.4	1.0
1,2	−0.1	−0.1	0.0	0.0
1,3	0.0	0.4	0.0	1.3
1,4	0.5	2.3	1.9	3.1
1,5	0.7	2.3	1.5	2.1
1,6	1.2	2.8	1.2	4.9
2,3	−0.1	—	0.2	0.3
2,4	0.7	1.5	2.9	9.0
2,5	1.2	4.2	3.2	8.2
2,6	2.6	3.1	5.6	8.5
3,4	0.7	0.6	2.0	1.8
3,5	1.5	3.1	4.1	5.1
3,6	2.8	3.8	5.2	4.9
4,5	1.3	1.6	4.4	1.8
4,6	−0.4	4.8	8.9	5.2
5,6	0.2	0.6	2.1	4.1

tiple crossover strands and the non-crossover strands. Double crossover strands are increased by 369 percent and triple crossovers by 1100 percent. The single crossover strands remain constant. The non-crossover strands are decreased by 41.7 percent (52.1 percent in the controls and 30.2 percent when both the Curly and Payne inversions are present).

The behavior of the tetrads in all four crosses was deduced by means of the following formulae for the distribution of crossovers in tetrads. (These have been derived by D. R. CHARLES [unpublished] from WEINSTEIN's generalized tetrad formulae [WEINSTEIN 1932 and 1936].)

$$
\begin{aligned}
T_{\cdot ijkl} &= 16s_{\cdot ijkl}\\
T_{\cdot ijk} &= 8(s_{\cdot ijk} - 4s_{\cdot ijkl})\\
T_{\cdot ij} &= 4(s_{\cdot ij} - 3s_{\cdot ijk} + 6s_{\cdot ijkl})\\
T_{\cdot i} &= 2(s_{\cdot i} - 2s_{\cdot ij} + 3s_{\cdot ijk} - 4s_{\cdot ijkl})\\
T_{\cdot o} &= s_{\cdot o} - s_{\cdot i} + s_{\cdot ij} - s_{\cdot ijk} + s_{\cdot ijkl}
\end{aligned}
$$

Where $T_{\cdot ijkl}$ = all tetrads with four crossovers regardless of the regions involved; $T_{\cdot ijk}$ = all tetrads with three crossovers regardless of the regions involved etc. and where $s_{\cdot ijkl}$ = all strands with four crossovers regardless of the regions involved etc.

Distribution of tetrads with crossovers in specific regions:

$$
\begin{aligned}
T_{ijkl} &= 16s_{ijkl}.\\
T_{ijk.} &= 8(s_{ijk.} - s_{ijk.l})\\
T_{ij.} &= 4(s_{ij.} - s_{ij.k} + s_{ij.kl})\\
T_{i.} &= 2(s_{i.} - s_{i.j} + 3s_{i.jk} - s_{i.jkl})
\end{aligned}
$$

Where $T_{ijkl.}$ = all tetrads which are crossovers in the specific regions ijkl only and $T_{ijk.}$ = all tetrads which are crossovers in the specific regions ijk only, etc., and $T_{ijk.l}$ = all tetrads which are crossovers in the specific regions ijk and one other region, l, and where $s_{ijkl.}$, $s_{ijk.}$, etc., have the same meaning for strands as $T_{ijkl.}$, etc., has for tetrads.

The tetrad computations of the data from the Curly cross indicate that those tetrads which show no crossovers in the portion of the X chromosome under observation (T_o in table 4) are greatly reduced in number and those tetrads which show one crossover ($T_{\cdot i}$ in table 4) are slightly reduced in number as compared with the controls. The numbers of tetrads showing two crossovers is greatly increased. In the cross with the Payne inversions, the decrease in non-crossover tetrads is not as great as that in the cross with the Curly inversions, but the effect on the single crossover and double crossover tetrads, while in the same direction as that caused by the Curly inversions, is far greater. As is to be expected, the combined inversions have a much greater effect than either of the inversions alone. They reduce the non-crossover tetrads to 4.7 percent of the total and single crossover tetrads to 16.4 percent while they increase the double crossover tetrads to 59.6 percent and the triple crossover tetrads to 19.2 percent.

Table 3 shows a comparison of tetrads having a crossover in a given region with those having a crossover in the given region plus one other region. In the controls we find that most of the crossing over in all six regions comes from single crossover tetrads (tetrads having only one crossover, that crossover being in the given region). In the cross with the Curly inversions, in regions one and six most of the crossing over occurred in $T_{i.j}$ tetrads (tetrads with two crossovers, one of which is in the given region i). In region 5 about the same amount of crossing over occurred in $T_{i.}$ tetrads as in $T_{i.j}$ tetrads. In the remaining regions most of the crossing over took place in $T_{1.}$ tetrads. In the cross involving the Payne inversions, most of the crossing over took place in the $T_{i.j}$ tetrads. This is the reverse of what took place in the controls. Finally, in the cross with the combined inversions, not only did most of the crossing over take place in $T_{1.j}$ tetrads, but these tetrads exceeded the $T_{i.}$ tetrads by at least 100 percent in every region. In regions 1, 2, and 6 the excess of $T_{i.j}$ tetrads over $T_{i.}$ tetrads is tremendous (table 3). Note the great reduction in the number of tetrads which crossed over in region 2 only.

TABLE 5

Showing the numbers and types of ♂ offspring of each of the four different classes of ♀ ♀ listed below

REGIONS	$\frac{+}{+};\frac{+}{+};\frac{y\ cv\ v\ f}{ec\ ct^6\ g^2}$		$\frac{+}{+};\frac{+}{Cy};\frac{y\ cv\ v\ f}{ec\ ct^6\ g^2}$		$\frac{+}{Payne};\frac{+}{+};\frac{y\ cv\ v\ f}{ec\ ct^6\ g^2}$		$\frac{+}{Payne},\frac{+}{Cy},\frac{y\ cv\ v\ f}{ec\ ct^6\ g^2}$	
	y	*non-y*	*y*	*non-y*	*y*	*non-y*	*y*	*non-y*
0	1033	1019	640	573	861	671	254	216
1	44	35	59	87	39	46	21	50
2	118	122	102	99	122	123	67	40
3	137	107	96	85	109	118	52	59
4	228	231	161	161	230	208	76	90
5	173	194	124	113	186	156	58	58
6	145	163	97	108	171	124	81	50
1, 2	0	0	0	0	0	1	1	1
1, 3	0	1	0	3	0	0	3	5
1, 4	2	3	8	9	6	12	10	12
1, 5	2	5	8	8	5	10	6	12
1, 6	6	6	10	9	6	6	10	15
2, 3	0	0	0	0	1	1	1	2
2, 4	4	4	8	5	15	14	24	22
2, 5	7	6	20	10	16	23	24	13
2, 6	13	15	16	6	26	29	23	18
3, 4	4	5	4	0	9	9	8	8
3, 5	10	5	13	9	22	16	11	13
3, 6	15	15	13	14	19	29	14	12
4, 5	7	7	6	6	22	22	13	8

TABLE 5 (*Continued*)

Showing the numbers and types of ♂ offspring of each of the four different classes of ♀ ♀ listed below.

4, 6	24	11	18	16	42	42	19	23
5, 6	2	2	1	4	10	17	4	9
1, 2, 3	0	1	0	0	0	0	0	1
1, 2, 4	0	0	0	1	0	0	0	0
1, 2, 5	0	0	0	0	0	0	1	0
1, 3, 5	0	0	0	0	0	0	0	2
1, 4, 5	0	0	0	0	0	0	1	4
1, 4, 6	0	0	0	0	0	2	1	3
1, 5, 6	0	0	0	0	1	0	1	0
2, 3, 4	0	0	0	0	0	0	1	0
2, 4, 5	0	0	1	0	2	1	1	2
2, 4, 6	1	0	1	0	2	0	2	5
2, 5, 6	0	2	0	0	3	2	1	0
3, 4, 5	0	0	0	0	0	0	0	1
3, 4, 6	0	2	0	0	0	0	4	2
3, 5, 6	0	0	1	0	1	0	0	0
4, 5, 6	0	1	0	0	2	0	2	2
1, 2, 4, 6	0	0	0	0	0	1	0	0
2, 4, 5, 6	0	0	0	0	1	0	0	0
1, 3, 4, 5, 6	0	0	0	0	0	0	1	0
Totals	1975	1962	1407	1326	1929	1683	796	758
Grand totals	3937		2733		3612		1554	

SUMMARY

The effects of autosomal inversions on crossing over in the first chromosome were measured. Four different crosses were made: (1) controls (no inversions in the autosomes); (2) Curly inversions in the second chromosome, third chromosome normal; (3) Payne inversions in the third chromosome, no inversion in the second chromosome; (4) Curly inversions in the second chromosome and Payne inversions in the third chromosome simultaneously. In all four crosses crossing over was measured in the first chromosome by means of the alternated 7ple gene complex.

Autosomal inversions increase crossing over in the X chromosome. This increase is accompanied by a great increase in multiple crossing over. These results confirm those of SCHULTZ and REDFIELD on the negative correlation of the interchromosomal effect with the intrachromosomal effect of inversions on crossing over.

The Curly inversions have their greatest effect on the yellow end of the X chromosome. This effect decreases rapidly until the *cv-ct*^{6} region is reached and thereafter it remains constant. The Payne inversions have an approximately uniform effect on crossing over throughout the portion of the X chromosome marked in this experiment.

The combined inversions have an effect similar to that of the Curly inversions, that is, greatest in the *y-ec* region, and constant to the right of the *cv-ct*^{6} region. This effect is greater in all regions than that of either the Curly or Payne inversions when used singly.

The total increase in map length of the *y-f* interval caused by the inversions was greatest in the cross with the inversions combined and least when the Curly inversions were used alone.

Analysis of the strand data shows that while there was a decrease in non-crossover strands and an increase in multiple crossover strands; the single crossover strands, with the probable exception of those in the cross involving the Cy chromosome alone, remained constant. Here again the order of magnitude of the effects of the inversions on the strand data was Cy; Payne > Payne > Cy.

Tetrad analysis showed that there was a reduction in non-crossover and single crossover tetrads and an increase in multiple crossover tetrads. As in the above two cases the order of magnitude of the effects of the inversions was Curly; Payne > Payne > Curly.

The author wishes to acknowledge his indebtedness to Professors L. C. DUNN and D. E. LANCEFIELD and to Mrs. JEANNE C. MOSSIGE for their many helpful suggestions and to Mr. JACK GODRICH for preparation of figure 1.

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SOMATIC CROSSING OVER AND SEGREGATION IN DROSOPHILA MELANOGASTER*

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INTRODUCTION

In 1925 Bridges found that females of *Drosophila melanogaster* containing the dominant factor Minute-n in one X chromosome and some recessive genes in the other X chromosome often exhibit a mosaic condition. While the main surface area of these flies showed the effect of the dominant Minute-n (I, 62.7) without the effect of the recessive genes, as was to be expected, smaller areas, in different regions of the body and of varying size, were not Minute-n, phenotypically, but displayed the effects of the recessive genes. Bridges' interpretation was this: the Minute-n factor has the property to eliminate occasionally the X chromosome in which it itself is located. The cells of mosaic spots are descended from one common ancestral cell in which such elimination had taken place. They possess, therefore, only one X chromosome and show the phenotype produced by its genes.

Minute-n is only one of a group of factors which are very similar in their phenotypical expression. The "Minutes" behave as dominants whose most striking phenotypic effect is a reduction in bristle size; in addition there is a strong retardation in development, tendency to rough eyes, etc. The homozygous Minute condition is lethal. Some Minutes have been shown to be deficiencies (Schultz 1929). Many Minute factors have been found in different loci of all chromosomes. They are distinguished by adding different letters or numbers to the symbol *M*.

Following Bridges' discovery of mosaics with respect to sex-linked factors, the appearance of mosaic spots which exhibit autosomal characters was described (Stern 1927b). Such spots appear on flies which originally had a constitution heterozygous for genes determining the characters. These mosaics occurred in crosses in which autosomal Minute factors were present and the facts seemed to agree with the interpretation that the spots were due to an elimination of that arm or part of an autosomal chromosome which carried the Minute.

The present investigation was originally designed to attack the problem: How is a Minute factor able to eliminate the chromosome or that part of a chromosome in which it itself is located?

At the same time the solution of another problem was sought. The fact that small mosaic spots showed the phenotypic effect of certain genes contained in their cells whereas the remainder of the individual showed another phenotype was proof of the autonomous development of these characteristics. Among the very few genes which did not show phenotypical effects in spots was the recessive "bobbed" (I, 66.0) which produces short bristles: in $+^{y}Mn+^{bb}/y+^{Mn}bb$ females the $y+^{M}$ spots did not possess the *bb*-type bristle length, but a $+^{bb}$ length. Non-autonomous development of the bobbed character seemed improbable as typical gynandromorphs had shown clear demarcation lines for the *bb* and $+^{bb}$ areas (STERN 1927a). As bobbed is located at the extreme right end of the genetic X chromosome, next to the spindle fibre attachment, the following hypothesis was proposed: just as in the case of autosomal eliminations only part of the autosome disappears, so also in *Mn* mosaics merely a portion of the X chromosome is eliminated. The piece adjoining the spindle attachment and including the bobbed locus is assumed to be left in the cell, thus giving a constitution $+^{bb}/y+^{Mn}bb$, which, being heterozygous for *bb*, does not produce the effect of this gene (STERN 1928b). When PATTERSON (1930) using MULLER's Theta translocation showed that in his cases of X-radiated flies "not the whole X chromosome was eliminated" it was decided to use the same genetic technique to test the above hypothesis as to the partial elimination in the case of *Mn*. The Theta translocation was kindly put at my disposal by Prof. H. J. MULLER.

Both problems, the question as to the action of Minutes to bring about elimination and the question as to complete or partial elimination of the X chromosome (intimately bound up with the question as to autonomous or non-autonomous development of bobbed in small spots), proved to be based on an erroneous concept as to the origin of mosaic spots. While the investigation revealed this, it provided at least a partial solution of the problems by the discovery of somatic crossing over and segregation in Drosophila.

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METHODS

The methods were similar to those used by BRIDGES. Flies were made heterozygous for recessive genes whose phenotypic effects were of such a kind as to be exhibited by very small areas, preferably even single setae. The setae of Drosophila are divided into macrochaetae and microchaetae, the former generally called bristles, the latter, hairs. As far as the purpose of the present study is concerned the distinction is of no intrinsic importance. Genes mainly used were: (a) yellow body-color (*y*, 1, 0.0), producing an effect which can be distinguished in a single hair, making it yellowish-brownish as opposed to the black not-yellow condition (the general coloring effect of *y* on the hypodermis is often not very distinct in spots (STURTEVANT 1932)) and (b) singed-3 (sn^3, 1, 21.0), producing a thickened, curved or crooked condition of the setae, which generally can be distinguished in single hairs also. However, doubts occasionally remain as to whether a single hair on a heterozygous $+/sn^3$ fly is genotypically singed or whether it is normal but slightly more bent than usual. With spots of two or more hairs such doubts hardly ever occur.

Following BRIDGES, the flies were inspected originally for spots only on the head and thorax. In later experiments, however, inspection of the abdomen was included. In order to discover even the smallest spots the flies were scrutinized under a binocular magnification of 37× (Bausch & Lomb objective 3.7, eyepieces 10×). The use of a simple device made by the Bausch & Lomb Optical Company which allows for the fine adjustment to be made by foot movements and leaves both hands free for manipulation, proved to be of great value (for more detailed description see Drosophila Information Service 6:60).

As the study of spots was practically confined to setae-bearing regions, a table of the number of setae on different parts of the body of average sized females was computed. Generally only the dorsal and lateral parts of the thorax and only the tergites of the abdomen were inspected and, except in special cases, no effort was made to remove the wings in order to uncover completely the median part of the abdominal tergites. This partly covered region excluded about 20 per cent of the abdominal setae from inspection (table 1).

A separate record was kept for each spot consisting of an outline drawing in case of head and thorax mosaics or of a notation of number of macro- and microchaetae and position in case of abdominal mosaics.

Spots on the dorsal side of the thorax nearly always form one single clearly defined mosaic area, while spots on abdominal tergites are fre-

TABLE I

Number of setae on different body regions.

	HEAD*	THORAX*	ABDOMINAL TERGITE					
			1 AND 2	3	4	5	6	7
Mean number of setae	34	238	108	120	117	123	120	61
Not inspected (estimate) %	0	0	50	40	20	0	0	0
Inspected	34	238	54	72	94	123	120	61
% of total inspected setae	4	30	66					
Inspected abdominal setae (% of total)			10	13	17	24	24	12

Total setae inspected ca 800
Total abdominal setae inspected ca 525.
* Dorsal parts only

quently broken up into two or more separate parts. Apparently the growth processes in the imaginal discs of thorax and abdomen are somewhat different.

THE ACTION OF MINUTE FACTORS

Blond-Minute

As stated in the introduction, a number of Minute determiners have been found to be deficiencies for short regions. The hypothesis suggested itself that the apparent tendency of Minutes to eliminate the chromosomes or chromosomal parts in which they are located is due to some mechanical disturbance of chromosome division which itself is caused by the material defect in the deficiency chromosome.

In order to test this hypothesis use was made of the "Blond-translocation" (Bld) which represents a reciprocal translocation between the left end of the X chromosome and the right end of the second chromosome (BURKART and STERN 1932). When all chromosomes of an individual are balanced with respect to the translocation, normal sized Blond bristles are produced. However, in females if one X chromosome lacks its extreme left end without being compensated for by the presence of the translocated piece on the second chromosome, then the individuals possess bristles of Minute, Blond character. BURKART had found that these Minute females show mosaic spots, a fact which seemed to indicate that elimination of the deficient X chromosome occurred.

The viability of Blond-Minutes is rather low and depends greatly on culture conditions. In half-pint milk bottles with one pair of parents their viability was found to average 30 per cent of their not-Minute sisters. Under Patterson's culture conditions the Minutes apparently never appeared and this led him to assume a viability factor in the region covered by the deficiency (Patterson 1932). The absence of this factor was believed to cause the death of the zygote.

In a Blond male the left end of the X is translocated to the right end of one of the second chromosomes. Such a male forms two kinds of X carrying gametes: (1) deficiency X, translocation II; (2) deficiency X, normal II. Mated to a normal female, two kinds of daughters are produced: (1) def./normal; transl. II/normal II; (2) def./normal; normal II/normal II. The females (1) are not-Minute, the females (2) are Minute. *If elimination of the deficient X chromosome is due to the deficiency itself then the percentage of eliminations should be equal in both classes.* Accordingly, Blond males were mated to females with morphologically normal chromosome constitution homozygous for singed-3. Elimination of the deficient chromosome was deduced from the appearance of not-Blond, singed setae mainly on head or thorax. Out of 323 Blond-Minute females (2) 143 had one or more such mosaic spots making a total of 167 (to which should be added 12 spots of somewhat different constitution cf. table 9). In 923 Blond not-Minute sisters (1) not a single spot was exhibited. This result proves that the deficient consitution of the Blond X chromosome itself is not the cause of eliminations, and suggests that eliminations of the X chromosome are due to the phenotypic "Minute reaction" (Schultz 1929), that is, to the same or part of the same physiological condition which results in the development of a short-bristled late hatching "Minute" fly.

One difficulty, however, remained. Why should the "physiological Minute condition" eliminate only the deficient X chromosome? That only the deficient X was affected and not its normal partner seemed to be indicated by an experiment in which the sn^3 gene was located together with Bld in the deficient X, the other X containing white (*w*, I, 1, 5) or other recessives. In 221 def. Bld sn^3/w; normal II/normal II Minute females, 94 bristles containing spots on head and thorax were found, all not-Bld, not-sn^3. They seemed to result from eliminations of the deficient chromosome, while the absence of sn^3 spots seemed to exclude the elimination of the not-deficient X chromosome. Was there such an interaction that the physiological Minute condition produced by an uncovered deficiency in the X would eliminate just this deficient chromosome which was quite stable when its phenotypic effect was suppressed by the normal allele for that region translocated to the second chromosome?

Autosomal Minutes and sex-linked spots

When the ability of autosomal Minutes to produce "autosomal" mosaics had been discovered, the following cross was made in order to detect a possible influence of such Minutes on the appearance of sex-linked mosaics: sn^3/sn^3 ♀ by $+^{sn}$; $My/+$ ♂ (*My*, Minute-y, III, 40.4). While 1020 $+^M F_1$ females had 3 single-bristle singed spots, 811 *My* F_1 sisters exhibited 16 spots (13 single-bristle, 3 larger ones, table 2a, first row). One of the spots in a $+^M$ fly occurred on the abdomen and is not included in the table. This result showed (1) that mosaic spots appear as rare occurrences even in not-Minute flies (BRIDGES in MORGAN, STURTEVANT, BRIDGES 1929, has encountered four such cases in his experiments) and (2) that an autosomal Minute increases the frequency of these occurrences, that is, is able to influence the fate of an X chromosome.

Later work confirmed this finding and has led to the use of autosomal Minutes as tools in the study of sex-linked mosaics.

Besides *My* three other autosomal Minutes have been tested for their effect on the X chromosome behavior, namely *Mw* (Minute-w, III, 80±), *M33j* (Minute-33j, III, 40.4) and *Mβ* (Minute-β, III, 85.4). Table 2a shows the results of some tests. It should be pointed out that the constitution of the X chromosomes varied in these experiments, so that the frequencies of spots in different experiments are not comparable. In some experiments only the head and thorax were inspected for spots. In all later tests the abdomen was inspected also. Due to the comparatively small size of the head and the low number of setae the number of head spots has been added to the number of thorax spots under one grouping. An inspection of tables 2a, b shows, among other results:

(1) The frequency of sex-linked spots in not-Minute flies varied from 0.0 to 6.0 on the head-thorax region and from 4.6 to 20.0 per cent on the abdomen. The frequency in Minute flies varied from 0.0 to 22.3 in the head-thorax region and from 8.0 to 36.6 per cent on the abdomen.

(2) A positive correlation is indicated between percentage frequency of head-thorax and abdominal spots:

(a) In not-Minute flies

experiment	*Mw*(5)	*Mβ*	*M33j*(3)	*M33j*(2)	*Mw*(4)	*M33j*(1)
frequency on head-thorax	0.0	0.7	0.8	1.4	4.5	6.0
frequency on abdomen	15.0	4.6	8.2	9.2	11.7	20.0

(b) In Minute flies

experiment	*Mw*(5)	*Mβ*	*Mw*(4)	*M33j*(3)	*M33j*(1)	*M33j*(2)
frequency on head-thorax	0.0	3.1	4.4	5.7	16.8	22.3
frequency on abdomen	27.1	8.0	28.1	33.4	36.6	15.9

Two of the discrepancies in these series seem to be based on a special condition which hindered the appearance of all head-thorax spots. This experiment (*Mw*(5)) therefore is not comparable with the rest.

(3) The relative increase of spots in Minute as compared to not-Minute flies varies from 1.0 to 15.6 times in the head-thorax and from 1.7 to 4.1 times in the abdominal region.

(4) The average increase is distinctly lower in the abdominal region.

(5) As far as the data are significant, no correlation seems to exist between amount of increase of spots in the two different body regions:

experiment	*Mw*(4)	*M33j*(1)	*Mβ*	*M33j*(3)	*M33j*(2)
increase on head-thorax	1.0	2.8	4.7	7.2	15.6
increase on abdomen	2.4	1.8	1.7	4.1	1.7

The data of tables 2a,b had shown the effect of autosomal Minutes on the *frequencies* of occurrence of sex-linked spots. Is there also an influence on *time* of occurrence in development of the process which leads to appearance of a mosaic spot? The earlier this time is, the larger the spot should be. Accordingly, in table 3 the spots are divided into three groups, those

TABLE 2a

Frequency of sex-linked spots in Minute and not-Minute females

MINUTE USED AND NO. OF EXP.	INDIVIDUALS INSPECTED		HEAD-THORAX SPOTS					ABDOMINAL SPOTS				
			NO.		%		%	NO.		%		%
	+	*M*	+	*M*	+	*M*	*M*.+	+	*M*	+	*M*	*M*.+
My†	1020	811	2	16	.2	2.0	10.5	—	—	—	—	—
Mw (1)†	964	813	8	23	.8	2.8	3.4	—	—	—	—	—
(2)†	377	284	7	17	1.9	6.0	3.2	—	—	—	—	—
(3)†	151	119	3	20	2.0	16.8	8.5	—	—	—	—	—
(4)	154	114	7	5	4.5	4.4	1.0	18	32	11.7	28.1	2.4
(5)‡	432	247	0	0	0	0	—	65	67	15.0	27.1	1.8
M33j (1)	135	131	8	22	6.0	16.8	2.8	27	48	20.0	36.6	1.8
(2)	349	251	5	56	1.4	22.3	15.6	32	40	9.2	15.9	1.7
(3)*	377	296	3	17	.8	5.7	7.2	31	99	8.2	33.4	4.1
Mβ	307	227	2	7	.7	3.1	4.7	14	18	4.6	8.0	1.7

† Abdomen not inspected.

‡ No head-thorax spots present.

* No head spots present.

TABLE 2b

Proportion of number of head-thorax:abdominal spots in $+^M$ and M females.

EXP.	NUMBER OF HEAD-THORAX AND ABDOMINAL SPOTS +	 *M*
Mw	7- 18	5- 32
M33j (1)	8- 27	22- 48
(2)	5- 32	56- 40
(3)	3- 31	17- 99
Mβ	2- 14	7- 18
Total	**25–116**	**107–237**

χ^2 about 10. P< 01.

comprising only one seta, only two setae, and more than two setae. This last class includes all spots from those involving three setae up to those occasional spots which include all setae of the imaginal disc concerned. The data are comparatively meager, but the following generalizations seem to be warranted:

(1) Most experiments agree in the relative frequencies of small and larger spots in that the one seta spots form the majority both on the head-thorax and on the abdominal region. (2) Some striking exceptions to this occur, notably the head-thorax spots in Minute flies of *Mw*(2) and (3) and the abdominal spots in both not-Minute and Minute flies of *M33j*(1). Here the frequencies of spots larger than one seta is higher than that of one seta spots: 14:3; 16:4; 20:7; 36:22. (3) The proportion of

TABLE 3

Frequency of sex-linked spots of different sizes in the experiments described in Table 2a.

EXP	HEAD-THORAX								ABDOMEN							
	NO. OF SPOTS		SIZE OF SPOTS						NO. OF SPOTS		SIZE OF SPOTS					
	+	*M*	+			*M*			+	*M*	+			*M*		
			1	2	>2	1	2	>2			1	2	>2	1	2	>2
My	2	16	2	—	—	13	1	2	—	—	—	—	—	—	—	—
Mw (1)	8	23	5	—	3	16	2	5	—	—	—	—	—	—	—	—
(2)	7	17	3	2	2	3	3	11	—	—	—	—	—	—	—	—
(3)	3	20	3	—	—	4	3	13	—	—	—	—	—	—	—	—
(4)	7	5	5	—	2	4	—	1	18	32	17	—	1	13	10	9
(5)	0	0	0	0	0	0	0	0	65	67	41	17	7	42	11	14
M33j (1)	8	22	6	1	1	13	4	5	27	48	7	6	14	22	11	25
(2)	5	56	5	-	—	42	4	10	32	40	17	4	11	25	6	9
(3)	3	17	3	—	—	16	—	1	31	99	19	6	6	60	17	22
Mβ	2	7	2	—	—	2	—	5	14	18	9	2	3	9	2	7

single seta spots is lower in the abdomen than in the head-thorax region. (4) In most experiments no striking influence of the Minute on the time of occurrence of the spot-producing process can be found.

The ontogenetic meaning of some of these findings will be dealt with later.

The specificity of the effects of sex-linked and autosomal Minutes

In view of the effect of autosomal Minutes on the X chromosome it seemed desirable to test for a possible influence of an X chromosome Minute on the behavior of autosomes. The Blond Minute is known to be one of the most potent factors for the production of sex-linked spots and accordingly was chosen for the test in regard to autosomal spots. Females of the constitution *h st cu sr* e^s *ca* (located over most of the length of chromosome III) were mated to Blond males and their Minute daughters were inspected for a condition mosaic for *h*, *st*, *sr*, e^s or *ca*. Although the 413 F_1 females exhibited on head and thorax 94 mosaic conditions for Blond, none was found to possess an autosomal spot. This shows that the influence of Blond-Minute on the production of autosomal mosaics must be very slight, if it exists at all.

This fact is significant. For if one makes a general "physiological Minute condition" responsible for the occurrences of spot-producing processes one might expect to find a corresponding seriation of different Minutes in respect to their potencies to produce both sex-linked and autosomal spots. However, a seriation in respect to frequency of sex-linked mosaics would show roughly:

$$\text{Blond-Minute} > Mn > M33j,\ Mw,\ My$$

while the same Minutes (*Mn* excluded) with respect to frequency of autosomal mosaics probably would have to be arranged as:

$$Mw,\ My > M33j,\ \text{Blond-Minute}$$

The two seriations are given after an analysis of the data presented in different tables of this paper. Too great reliance cannot be attributed to details of these arrangements, as the experiments were carried out over a period of years and under different genetic and environmental conditions. Therefore no special table has been made up from these data, as the quantitative results might indicate a higher degree of accuracy than they really represent. However, there seems no doubt as to the validity of the main result, namely that the effect of Minutes on the frequencies of sex-linked and of autosomal spots varies independently so that one Minute factor may affect strongly the number of sex-linked but only slightly the number of autosomal spots and vice versa.

An even more striking correlation between certain autosomal Minutes and areas mosaic for definite regions of the same autosome will be presented in the chapter on "Autosomal spots."

THE MECHANISM OF MOSAIC FORMATION

Various hypotheses

In the foregoing pages the effect of Minutes on the process of mosaic formation was discussed in general terms. The following part will contain an analysis of the process itself.

BRIDGES' work with females carrying *Mn* in one X and recessive genes in the other seemed to have established (1) that the cells of a spot do not contain the *Mn* chromosome and (2) that these cells are male in constitution, containing only one X with the recessive genes. Three main possibilities suggested themselves as mechanisms for the elimination of the *Mn* chromosome from the cells of the spot: (a) during a somatic division the *Mn* X chromosome does not divide and consequently passes into one daughter nucleus, leaving the other one in possession of only a division product of the not-*Mn* X chromosome; (b) the *Mn* X chromosome divides into two halves, but only one half passes into one of the daughter nuclei. The other half lags behind, is not included into a daughter nucleus, and degenerates in the cytoplasm; (c) the *Mn* X chromosome divides into two halves. These halves do not disjoin but pass together into one daughter nucleus.

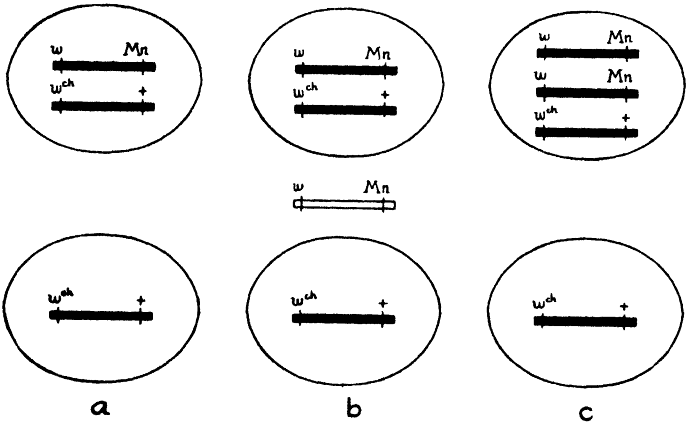


FIGURE 1 a–c. Three possibilities to account for elimination of an X chromosome.

The constitution of the daughter nuclei according to the three hypotheses is pictured in figure 1. No way of distinguishing between the mechanisms (a) and (b) was found, but a test between (a) and (b) on one side and (c) on the other seemed possible. The sister cell of the "elimination cell" in (a) and (b) would be of the same constitution as the original constitution of the fly, while in (c) it would have a different constitution, being triplo-X. By using suitable gene markers such a condition as (c) could be demonstrated if it occurred. Accordingly females were bred which had white in their *Mn* chromosome and cherry (w^{ch}) in the other X chromosome. The eye color in such females is a light cherry. Their eyes were searched for spots of normal, dark cherry color, indicating the elimination of the *w Mn* chromosome. When such spots were found it was determined whether their surroundings in the eye were of the w/w^{ch} coloration as expected according to (a) and (b) or whether they contained a very light cherry spot, indicating the $w/w/w^{ch}$ constitution expected according to (c). On 1702 $w\ Mn/w^{ch}$ females which were inspected 20 eye spots were found. They all were cherry colored and unaccompanied by a twin spot of $w/w/w^{ch}$ coloration or of the irregular facet arrangement characteristic for $3X+2A$ eyes. Sixteen of these spots covered an area of at least 3 or 4 but not more than about 50 facets; 4 spots included more than 150 and less than 300 facets. These facet numbers have not been determined accurately but have been estimated from sketches. The total number of facets in one eye is between 700 and 800 in females. Similarly, in 168 $w^{ch}Mn/w$ females, two white eye spots were observed not accompanied by a twin spot of the darker colored triplo-X constitution $w^{ch}Mn/w^{ch}Mn/w$. These results rule against the hypothesis (c) provided that cells with two *Mn* factors and one normal allele are viable and can give rise to cell patches large enough to be recognized as mosaic spots. Nothing was known originally about this point.

Somatic segregation

y/sn^3 flies; preliminary discussion

The solution was brought about by chance. When it had been found that the "Minute condition" caused by an "uncovered" Blond-deficiency was necessary for the elimination of the deficiency X chromosome, an experiment was made in order to determine whether a deficiency which in itself does not produce a Minute effect would, together with an independent Minute factor, bring about the elimination of the deficient chromosome. The deficiency chosen was the well known Notch-8 (N^8) in the X chromosome and the Minute was the autosomal *Mw*. The following cross was made: $N^8/y\ Hw$ dl-49 ♀ by sn^3; $Mw/+$ ♂, and the frequencies of head-thorax spots in N^8 and not-N^8 sister females were compared (table 4). The presence of *Hw* and of the dl-49 inversion is irrelevant. From 280 $N^8/sn^3;+^M$ or *Mw* females 9 mosaic spots were obtained while 381 control $y\ Hw$/dl-49/$sn^3;+^M$ or *Mw* females yielded 15 spots. Thus the frequency of spots in N^8 was 3.6 per cent, in controls 3.9 per cent. There was no interaction of the Notch deficiency with the autosomal Minute-w.

The nine spots in the N^8/sn^3 flies were recognized by singed setae occurring presumably as a consequence of elimination of the N^8 chromosome. In cases where the sn^3 containing X chromosome would have been eliminated, no visible spot would have been produced, for the N^8 chromosome contained no recessive genetic marker which would have expressed itself in a spot (provided that a cell containing only the Notch-deficient X chromosome is able to reproduce sufficiently to give rise to a large enough cell-patch).

TABLE 4

N^8/y Hw dl-49 by sn^3; Mw/+. Head and thorax spots only.

	N^8		$+^N$	
	$+^M$	*Mw*	$+^M$	*Mw*
y spots	-	--	1	1
sn^3 spots	4	5	0	2
y– *sn^3* twin spots	—	-	2	0
Total spots	**4**	**5**	**3**	**12**

The situation was different in the *y Hw* dl-49/*sn^3* control flies. If there was no preference which of the two X chromosomes would be eliminated, then two different types of spots might be expected to occur in about equal numbers: (1) *y* spots in case of elimination of the *sn^3* X chromosome and (2) *sn^3* spots in case of elimination of the *y Hw* dl-49 X chromosome. The 15 spots found consisted of (1) two *y* spots and (2) two *sn^3* spots while (3) the remaining 11 spots showed an unexpected structure: ***they were twin-spots formed by a yellow not-singed area adjacent to a singed not-yellow area.***

The obvious explanation is that, during a somatic division of one of the cells of these 11 *y Hw* dl-49/*sn^3* females, a segregation had taken place whereby one daughter cell obtained the yellow gene carried originally by one of the X chromosomes while the other cell obtained the singed gene carried originally by the other X. A process similar to gametic segregation of genes lying in opposite members of a pair of chromosomes had occurred in a somatic cell. The further division and normal somatic differentiation of the two daughter cells finally gave rise to mosaic twin areas.

These findings of somatic segregation suggested that the so-called chromosome elimination in certain cells leading to the appearance of mosaic spots was in all or most cases the consequence of somatic segregation. This theory is substantiated by three facts. (1) Further experiments demonstrated the general occurrence of twin spots in flies of suitable constitutions. (2) In appropriate experiments it could be shown that nearly all mosaic spots exhibit the results of somatic crossing over. This makes simple elimination hypotheses improbable. (3) The theory solves the difficulties encountered by the assumption that the sex-linked Minutes eliminate only their own chromosomes.

We shall first discuss point (3). The more important statements (1) and (2) will be dealt with in later sections.

Minute-n and Blond-Minute "elimination" as somatic segregation

Somatic segregation of the X chromosomes in a female carrying *Mn* in one X and a recessive gene in the other will lead to two daughter cells, one containing only *Mn*, the other only the recessive. *Mn* is known to be lethal to a male or a homozygous *Mn* female zygote. If we assume *Mn* in such a condition to be lethal to a somatic cell also we shall expect the one daughter cell to die while the other one, containing only the recessive, will give rise to the observed spot. (It might be argued that a cell containing only *Mn* is viable but phenotypically not different from the non-segregated surrounding tissue. This, however, is excluded by having a recessive gene together with *Mn* in the one X chromosome but not in the other X. Segregation without lethal effect of the *Mn* segregation product should exhibit *Mn* spots which also show the recessive gene effect. In the experiments discussed on p. 635 the *Mn* chromosome contained a white or cherry gene, but no spots showing the respective eye colors were found. Other experiments of similar nature are described in later sections of this paper.)

Somatic segregation can account also for the single spots in "Bld-Minute" flies. In heterozygous Bld-Minute females it will lead to a "not Bld-Minute" and a "Bld-Minute" cell. The former will give rise to a spot with not "Bld-Minute" phenotype while the latter is expected to die, that is, if one assumes a cell not to be viable in case it contains a completely uncovered X chromosome deficiency. Male zygotes containing the uncovered Bld-deficiency X chromosome or female zygotes homozygous for such a condition are known to be not viable.

The reader might be inclined to strengthen these arguments by pointing to DEMEREC's studies (1934) on cell lethals. This, however, would not be justified as DEMEREC's interpretation is *based* on the acceptance of the theory of somatic segregation and therefore cannot be used to prove this theory.

The influence of a Minute condition on the occurrence of mosaic spots thus consists in an increase of the tendency to somatic segregation. The question asked at the close of the chapter on mosaic spots in Bld-Minute flies can now be answered. "Is there an interaction of such a kind that 'the physiological Minute condition' produced by an uncovered deficiency in the X chromosome would eliminate just this deficient chromosome?" The answer is this: elimination is not restricted to one X chromosome, but somatic segregation of the two X chromosomes leads to the appearance of spots which possess the non-deficient chromosome only.

Further analysis of somatic segregation in y/sn^3 flies

In order to justify the assumption of somatic segregation as a general cause of the occurrence of mosaic spots we have here to deal with those cases of table 4 which did not show a twin condition although such a condition might have been expected, for whenever a cell contains in each of its two X chromosomes one or more recessive, heterozygous genes capable of producing their phenotypes in small surface spots, the simple process of segregation will yield two neighboring cells pure for the genes of their respective segregated chromosomes. Why then did only 11 out of 15 cases show the twin spot condition? The following considerations have to enter into an answer to this question. (a) Even under the assumption that somatic segregation always leads to two sister cells which are both pure for originally heterozygous recessive genes, the appearance of the mosaic region will depend on the time of the segregation process in ontogeny. With a very late occurrence in development only two small twin areas could be produced, each consisting of only one cell if segregation took place during the very last division of the cells of the imaginal disc. In such cases it will frequently happen that one of the segregation products will form a seta and thus be recognized by its singed shape or yellow color, while the other segregation product will not happen to build up such a part of the hypodermis as will give rise to a seta and thus will not be recognizable. In other words, small twin spots will be liable to be recognized only in one of the segregation products. In large spots the probability is correspondingly higher that the areas formed by both segregation products will contain setae. They should therefore show the twin condition. (b) Even in larger spots a twin condition will be found lacking in case the cell descendants of one of the segregation products happen to become located in a region bare of setae, as for instance most of the scutellum, large areas on the head, the sternopleurae, the regions laterally from the posterior dorsocentrals. Twinning will be absent also in cases where the survival or reproductive ability of one of the daughter cells of the segregating division has been impaired, be it by chance, by normal developmental determination, or by lower genetic viability. To sum up the preliminary discussion: Small spots are expected to be often "singles," not twins; large spots in the majority of cases should be twins.

Tables 5 and 6 contain data pertinent to this question. Four different but fundamentally similar groups of experiments are summarized. In experiment 1 the females were of the constitution *y Hw* dl-49/sn^3 with or without *Mw*. They include, together with others, the flies discussed at the beginning of the present chapter. It is seen (table 5) that 23 out of 38 spots were twins and that 10 out of the 15 single spots were so small as to include only one seta, which obviously makes it impossible for them to show a twin condition. Of the 28 spots covering two or more setae the great majority, namely 23, were twins. Experiment 1 then corresponds closely to our expectation. In experiment 2 the constitution of the flies was *y w*/sn^3 or *y* w^e/sn^3 (*w* and w^e will be disregarded here). In addition, all individuals contained *M33j*. There were 67 twin spots out of a total of 212, and 102 out of the 145 not-twin spots were single seta spots. Of the 110 spots covering two or more setae 67 exhibited the twin condition, and 43 did not. But 21 of these 43 were so small as to include only two setae, thus still making it probable that the supposed twin area did not happen to cover a setae-forming region. Although the results in experiment 2 did not come as near to expectation as in 1 the agreement can be regarded as sufficient. The results will be further discussed after a description of experiments 3 and 4. In these a high frequency of spotting was induced by making the flies homozygous for recessive, mutant alleles of bobbed. In 3 one allele was the standard *bb*, the other one either the same or a very similar one; in 4 one was the lethal allele bb^l the other was as in 3. Bobbed

TABLE 5

Kinds and sizes of spots in experiments involving primarily y and sn^3 when located in opposite chromosomes.

EXP.	CONSTITUTION		INDIVID.	SPOTS	*y* SPOTS			sn^3 SPOTS			*y*-sn^3 TWIN SPOTS	
					NO. OF SETAE			NO. OF SETAE			NO. OF SETAE	
					1	2	>2	1	2	>2	2	>2
(1)	*y Hw* dl-49/sn^3		551	38†	2	– –	2	8	1	2	4	19
(2)	*y w* (or w^e)/sn^3		376	212‡	40	9	12	62	12	10	7	60
(3)	*y* g^2bb/sn^3bb^x	(a)	635	6†	–		1	5		-	—	
		(b)	635	157*	22	8	6	60	14	9	9	29
(4)	*y bi cv* ct^6 *v*											
	g^2 bb^l/sn^3bb^x	(a)	214	19†	1	-	- -	5	4	8	—	1
		(b)	214	73*	16	4	1	12	7	15	3	15
					81	21	22	152	38	44	23	124
	Totals		**1776**	**505**	**124**			**234**			**147**	

† Head and thorax spots only
‡ Head, thorax and abdominal spots
* Abdominal spots only.

can be called a recessive Minute gene, so that the flies in these experiments were under the influence of a "physiological Minute condition," which caused the high spotting frequency. Again the presence of other genes besides y and sn^3 will be disregarded at this point. In 3 only 38 out of 163 spots were twins, but a consideration of the different sizes of spots again shows that 87 out of the 125 single spots were so small as to include only one seta. Of the remaining 38 single spots, 22 were so small as to include only two setae, but of the 76 spots covering two or more setae, there were 38 twin spots. Experiment 3 then, although showing a general agreement with expectation seems to deviate more from it than the two experiments 1 and 2. Before discussing this we shall consider experiment 4. Here 19 out of 92 spots were twins, and 34 out of the 73 single spots included only one seta. Of the remaining 39 single spots, 15 were so small as to include only two setae. Out of the total of 58 spots covering two or more setae, 19 exhibited the twin condition. As in experiment 3 these results seem to be in general, but not very close, agreement with the theory of simple somatic segregation as the cause of spotting.

TABLE 6

Further data on the size of spots in experiments 1–4 of table 5

	(1)	(2)	(3)	(4)
Total y setae in single spots	137	182	69	26
Total sn^3 setae in single spots	113	145	121	183
Total y setae in twins	121	230	64	27
Total sn^3 setae in twins	80	208	86	45
Average y setae in twins	5.3	3·4	1.7	1·4
Average sn^3 setae in twins	3·5	3.1	2.3	2·4
Average ($y+sn^3$) setae in twins	8.8	6.5	4·0	3·8

Table 6 summarizes certain facts which help to explain the apparent discrepancies. The last horizontal line shows that the average size of the twin spots in experiment 1 was more than double that of the twin spots in 3 and 4 while it was intermediate in 2, and that in 3 and 4 the average size of one of the twin areas was only of the order of magnitude of two setae. It follows that there was a considerably higher chance for the small twin areas in 3 and 4 to appear phenotypically only as single spots than there was for the large areas in 1 and 2. Part of the deviations from our expectation are further cleared up by the following considerations. If single spots are really parts of twin areas in which only one area had the opportunity to exhibit its phenotype, then if chance alone determined which of the two areas covered a seta-forming region one should expect an equal frequency of sn^3 and of y single spots. An inspection of table 5, however, shows that, at least in 3 and 4, there is no 1 : 1 ratio of the two types of spots. In different form this can be seen from the first two lines of table 6 where the total

TABLE 7

List of all 124 twin spots >2 from table 5 with number of y and sn^3 setae affected. Each horizontal line is the record of one kind of spot The numbers in parentheses indicate the frequency with which this type of spot was represented. No number in parentheses was given when there was only one spot of its kind.

EXP. (CF. TABLE 5)	NO. OF SETAE		DIFFERENCE NO. y–NO. sn^3
	y	sn^3	
(1)	3	1	+ 2
	28	1	+27
	(2) 1	2	− 1
	(3) 3	2	+ 1
	4	2	+ 2
	(2) 1	3	− 2
	3	3	0
	4	3	+ 1
	24	3	+21
	5	4	+ 1
	3	5	− 2
	5	5	0
	5	9	− 4
	1	14	−13
(2)	(7) 2	1	+ 1
	(2) 3	1	+ 2
	(2) 4	1	+ 3
	5	1	+ 4
	10	1	+ 9
	50	1	+49
	(4) 1	2	− 1
	(4) 2	2	0
	(3) 3	2	+ 1
	5	2	+ 3
	(5) 1	3	− 2
	(3) 2	3	− 1
	(2) 3	3	0
	4	3	+ 1
	(2) 5	3	+ 2
	(2) 6	3	+ 3
	(4) 1	4	− 3
	2	4	− 2
	(3) 3	4	− 1
(2) (cont'd)	(2) 1	5	− 4
	2	5	− 3
	4	5	− 1
	6	6	0
	1	7	− 6
	15	7	+ 8
	3	7	− 4
	1	10	− 9
	11	14	− 3
	1	15	−14
(3)	(5) 2	1	+ 1
	5	1	+ 4
	(6) 1	2	− 1
	(2) 2	2	0
	(2) 3	2	+ 1
	1	3	− 2
	(2) 2	3	− 1
	1	4	− 3
	(2) 2	4	− 2
	3	4	− 1
	4	4	0
	(2) 1	5	− 4
	2	5	− 3
	3	7	− 4
(4)	(2) 2	1	+ 1
	3	1	+ 2
	(5) 1	2	− 1
	2	2	0
	(3) 1	3	− 2
	3	3	0
	1	4	− 3
	2	5	− 3
	1	6	− 5

number of *y* and *sn*^{3} setae has been noted. It is evident that the *y* setae are in the minority in 3 and 4, while there is a deficiency of *sn*^{3} setae in 1 and especially in 2. Possible causes for these new discrepancies can be learned from the second part of table 6. Here the frequencies of sn^3 and *y* setae are listed for all twin spots, that is, for all cases in which there is no doubt as to the occurrence of somatic segregation. The relative frequencies of the two types of setae in twin spots vary in the same direction as the total frequencies in single spots. Obviously, in these experiments, the chances for survival and reproduction of the two daughter cells from a segregating division were not equal. The vitality of *y* cells was lower than that of sn^3 cells in 3 and 4 but higher in 1 and 2. The greater the inequality in survival value, the higher the proportion of spots even of larger sizes which should appear only as single spots, the twin area having died or been kept small. This is roughly borne out by the data. What the causes of lowered viability were in these experiments cannot be determined accurately now. The presence of different alleles of bobbed in the two X chromosomes of 4 and possibly 3 has nothing to do with it, as will be shown later. However, it is suggestive that the cells in 4 which are pure for *y* are also pure for *bi*, ct^6, *cv*, *v*, and g^2. As it is known that this multiple mutant condition considerably lowers the viability of a whole individual, the assumption seems justified that a similar effect may be found also in mosaic parts.

Table 7 has been added to give a more complete representation of all twin spots with more than 2 setae which occurred in these experiments. It is interesting that such great inequalities of the two twin areas were observed as the case in which *y* area covers 50; sn^3 area covers 1; or *y* area has 24 setae and sn^3 area 3; or *y* area 1 and sn^3 area 15 seta.

Some of these very unequal twin spots might possibly be regarded as two single spots of independent origin, lying next to each other, but this must be very exceptional. The frequency of spots in most experiments is low enough as to make rare the occurrence of more than one spot on an individual, although the incidence of flies with two or more spots is higher than according to chance (BRIDGES in MORGAN, STURTEVANT and BRIDGES 1929; also numerous data of the author). If several spots occur on one fly they have no tendency to be neighbors.

Summing up, it seems clear that somatic segregation does not only account for the occurrence of twin spots but also for that of single spots. But while the evidence in the cases of twin spots is direct, in the cases of single spots it is of such a nature as to leave open the possibility that not all single spots can be regarded as vestigial twin spots whose region did not happen to affect a seta. A numerical treatment of the data which theoretically should be able to give a final decision is not feasible on account of the many variable factors involved. The next section, however, will show that a certain proportion of single spots are to be expected which have never been partners of an original phenotypic twin group.

Somatic segregation and crossing over

Experiments involving *y* $sn^3/+$ flies

In the experiments summarized in table 5, *y* and sn^3 were in opposite chromosomes. When both mutants were in the same chromosome, some new results were obtained (table 8): Three kinds of spots appeared with different frequencies, namely 110 *y* sn^3, 43 *y* and 7 sn^3 spots. The finding of *y* sn^3 spots was expected, for segregation in *y* $sn^3/+$ females (disregarding the presence of *bb* and *car*) should give rise to *y* sn^3 and + cells, which would be visible as *y* sn^3 spots. The occurrence of *y* and of sn^3 spots needs an additional interpretation. Somatic crossing over between *y* and sn^3 would separate these two genes from each other and thus afford an explanation. If the crossover process occurred during a two strand stage, the resulting strands would be $y+^{sn}$ and $+^{y}sn^3$, and if segregation ensued a *y* and a sn^3 twin spot would be produced. No twin spots were found, making the two strand crossing over assumption invalid. If, however, somatic crossing over occurred at a four strand stage between two of the four strands, and segregation two strands by two strands followed, then the facts can be explained (fig. 2). It is seen that following single crossing over, different types of chromatid segregation, namely **x** and **y**, give rise to either *y* or sn^3 single spots. (Throughout this paper the term "chromatid" is used in reference to the strands which *constitute* a multivalent chromosome group during prophase and metaphase, as well as in reference to those chromosomes of anaphase and telophase which *originated* from a multivalent.)

TABLE 8

Spots in flies of the basic constitution y $sn^3/+$.

EXP.	CONST	IND	SPOTS	*y* sn^3			*y*			sn^3			OTHER SPOTS
				1	2	>2	1	2	>2	1	2	>2	
(1)	*y* $sn^3bb/car\ bb$	83	34	10	4	6	3	3	5	2		1	—
(2)	*y* $sn^3bb/car\ bb$	495	72	25	15	10	12	2	5	2	—	1	—
(3)	*y* $sn^3bb/+$†	508	35	10	11	4	5	4		1	—	—	—
(4)	*y* $sn^3/+$†	321	21	8	3	4	1	1	2	—	—	—	2*
				53	33	24	21	10	12	5	—	2	
Totals		1407	162		110			43			7		2

† Partly $Mw/+$.

* $+^{y}+^{sn}$; ♂-colored.

If crossing over occurs at the four strand stage the subsequent segregation process will be expected to lead to either one of two results. (1) A separation of the four chromatids into two daughter cells will occur, followed

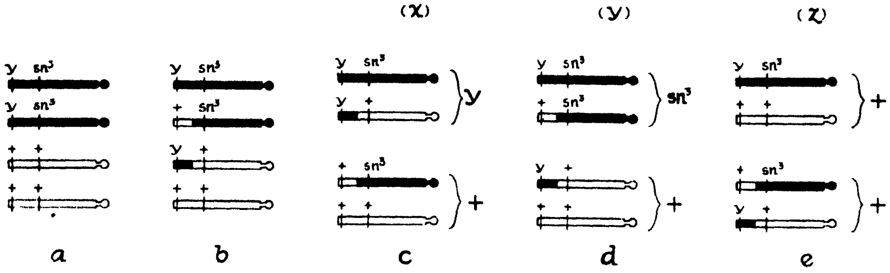


FIGURE 2. $y\ sn^3/+$ Crossing over between y and sn^3 at a four strand stage a. Non-crossover chromatids b Two crossover and two non-crossover chromatids c-e Three different types of chromatid segregation

later by normal mitosis or (2) the initiated segregation will bring about a true reduction of chromatids leading to a second segregating division with resulting cells containing only single X chromatids. In females of the constitution $y\ sn^3/++$, such a reduction process, after crossing over between two of four strands, should lead to four cells with the genes $y\ sn^3$, $y+^{sn}$, $+^{y}sn^3$ and $++$. The areas resulting from later cell-divisions of the segregation products would exhibit the phenotypes $y\ sn^3$, y, and sn^3. The visible result would be a triple spot. Not a single spot of this nature was observed; the hypothesis of a complete somatic reduction process is thus refuted.

We can test the assumption of somatic crossing over at a four strand stage by applying it to the earlier experiments in which the constitution of the flies was y/sn^3. Single crossing over between y and sn^3 at a two strand stage would yield $y\ sn^3$ and $++$ strands; segregation would result in $y\ sn^3$ spots. No such spots have been found (table 5). Crossing over at a four strand stage, however, would, according to different types of chromatid segregation, produce y or sn^3 single spots (fig. 3). Such spots did occur and while many of them could be regarded as vestiges of potential twin spots, it is possible to assume a certain number of them to have been products of crossing over between y and sn^3 in the manner suggested.

Again it is obvious that no reduction of chromatids occurred which

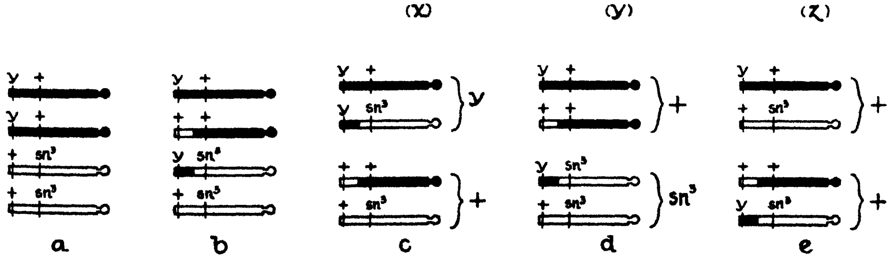
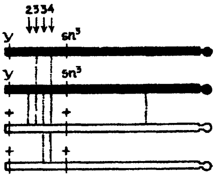


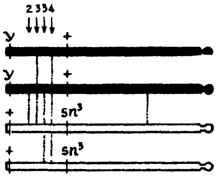
FIGURE 3. y/sn^3. Crossing over between y and sn^3 at a four strand stage.

would have resulted in triple spots *y*, *sn*^{3}, and *y sn*^{3}. No triple spots were found and the phenotype *y sn*^{3} did not occur even in single spots.

If one characterizes the two types of chromatid segregation **x** and **y** by the constitution of the right chromatid ends, then **x**-segregation would be equational and **y**-segregation reductional. As the right end of the X chromosome is known to contain the fibre attachment point, processes **x** and **z** would result from separation of sister attachment points while **y** would occur only when sister points stay together. It is possible to account for all observed spots with the assumption of equational segregation for the



strands involved		(x)	(y)	(z)
2	y sn³ y + + sn³ + +	sn³ +	y +	+ +
3	+ sn³ y + y sn³ + +	sn³ +	+ +	+ y
3	y sn³ + + + sn³ y +	sn³ +	+ +	y +
4	+ sn³ + + y sn³ y +	sn³ +	+ y	+ +

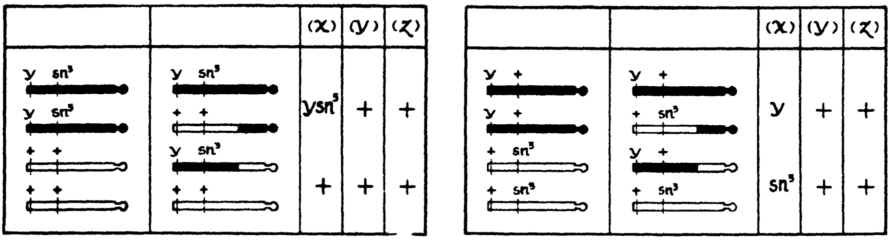


strands involved		(x)	(y)	(z)
2	y + y sn³ + + + sn³	+ sn³	y +	+ +
3	+ + y sn³ y + + sn³	+ sn³	+ +	+ y
3	y + + sn³ + + y sn³	+ sn³	+ +	y +
4	+ + + sn³ y + y sn³	+ sn³	+ y	+ +

FIGURES 4 (LEFT) AND 5 (RIGHT). *y sn*^{3}/+ and *y*/*sn*^{3}. The four different types of double crossovers, involving 2, 3, and 4 chromatids and the results of the three different types of segregation.

right end, if one considers the possibility of occurrence of somatic double crossovers simultaneously to the left and to the right of *sn*^{3} (figs. 4, 5). Four different kinds of double crossover processes within the four strand "tetrad" are possible, involving two, three, and all four strands. After single crossing over **x**-segregation results in *y* single spots in flies of both constitutions $y\ sn^3/++$ and $y+/+sn^3$, after double crossing over it results in *sn*^{3} spots. If **y** segregation occurs, it gives rise to *y* spots in one-half of all cases; similarly **z** segregation produces visible *y* spots in two out of the four cases.

While a decision between the hypotheses of single crossing over and different kinds of segregation or single and double crossing over and equational **x**- and **z**-segregation only cannot be derived from the present data, the second hypothesis seems to be in better agreement with the known facts in regard to the separation of daughter chromosomes in mitosis. We shall return to this question in the next chapter.



FIGURES 6 (LEFT) AND 7 (RIGHT) $y\ sn^3/+$ and y/sn^3 Results of crossing over to the right of *sn*^{3}.

What is the frequency of the different crossover types in the $y\ sn^3/+$ experiments? We shall regard (and shall justify this hypothesis later) the *y sn*^{3} spots as due to single crossing over between *sn*^{3} and the right end of the X chromosome (fig. 6; see also fig. 7 for the y/sn^3 flies). The *sn*^{3} spots are considered to be due to double crossing over. In determining the frequency of crossing over we cannot follow the usual procedure in germinal crossing over and base the calculation on the total of observed non-crossovers and crossovers, since the absence of crossing over in the great majority of somatic cells makes the number of non-crossovers rather meaningless. However, we can determine the relative frequencies of observed crossovers to the right and to the left of *sn*^{3} and the frequency of double crossovers. The frequencies of *y sn*^{3}, *y*, and *sn*^{3} spots were 110, 43, and 7. As was shown earlier, some *y* spots have to be regarded as products of **z**-segregation after double crossing over. As they constitute one-third of all visible double crossover products, their number is taken as 3.5. After subtracting this number from the total number of *y* spots, we find the proportion of spots produced after crossing over to the right of *sn*^{3} (*y sn*^{3} spots) to spots from crossing over to the left of *sn*^{3} (*y* spots) to be 110:39.5. Only the **x**-type of segregation in single crossing over yields visible spots, while the equally frequent **z**-type remains undetected. In double crossing over both **x**- and **z**-segregations result in visible spots, a total of 75 per cent of all cases. In order to have comparable figures we have to insert two-thirds of the number of visible double crossover spots, namely $(7+3.5)\cdot\frac{2}{3}=7$. Thus the proportion, single crossovers to the right of *sn*^{3}:singles to the left:doubles is 110:39.5:7. Comparing these values with the corresponding frequencies in meiosis, we find for the single crossovers 36:20.5. (MORGAN, BRIDGES, and STURTEVANT 1925, modified; no satisfactory data are available for the double crossovers.) In mitosis there seems to occur a higher proportion of all crossovers to the right of *sn*^{3} than in meiosis. Even this frequency of somatic crossovers to the left of *sn*^{3} was unusually high, as further experiments show.

Experiments involving Blond

In table 9 experiments are summarized in which somatic crossing over in females heterozygous for Blond, and either *sn*^{3} or a forked (*f*, 56.7) allele was studied. The flies had two normal chromosomes II and were therefore of Minute type on account of the Blond deficiency in the X chromosome. In experiment (1) 167 out of 179 spots can be explained as

TABLE 9

Spots in Blond-Minute flies heterozygous for sn^3, f, or f^5, mainly head-thorax spots except in exp. (2)

EXP.	CONST.	SPOTS	TYPE OF SPOT*		
			$+^{Bld}+^{m}+^{M}$	$+^{Bld}m+^{M}$	$+++\ +f^5+$
(1)	$\frac{Bld\ +}{+\ sn^3}$	179	9(+3†)	167	—
(2)	$\frac{Bld\ +}{+\ f^5}$	178	8	168	2
(3)	$\frac{Bld\ sn^3}{+\ +}$	91	91	—	—
(4)	$\frac{Bld\ f}{+\ +}$	15	15	—	—

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* *m* stands for either *sn*^{3} or *f*; $+^M$ for "not Bld-Minute," i.e., long setae; "–" signifies a twin spot condition ($+++$ next to $+f^5+$).

† The three spots had $+^{Bld}+^{sn}$ setae of still smaller length than Bld-Minute.

products of crossing over to the right of sn^3, for if such crossing over occurs, normal equational disjunction of daughter fibre points will produce two sister cells of the constitution Bld+/Bld+ and $+sn^3/+sn^3$. The former is assumed not to be able to give rise to a viable patch of cells, the latter multiplies to produce a $+^{\mathrm{Bld}}$ sn^3 spot with normal sized setae. As can be readily seen from constructing a diagram for this case similar to our figure 7 for a previously discussed experiment, the other two mathematically possible types of disjunctions **y** and **z**, do not give rise to visibly new constitutions. However, crossing over to the left of sn^3 and subsequent disjunction of sister attachment points (fig. 3) can lead to sister cells Bld+ /Bld sn^3 and ++/+ sn^3 the latter of which result in normal sized, not-Bld, not-sn^3 bristles. Nine such spots were found. Non-disjunctional segregation **y** of the fibre points would produce Bld $sn^3/+sn^3$ bristles. No such case was found. Double crossovers which after normal disjunction of the fibre points (similar to figure 5) also would yield Bld $sn^3/+sn^3$ spots besides half as many wild type spots were also absent. (There were three spots with not-Bld, not-sn^3, but shorter than Bld-Minute bristles. Their nature is not clear.)

In summing up experiment 1, we find the great majority of spots 167 out of 179, to be due to crossing over to the right and only 9 due to crossing over to the left of sn^3. This is a much lower proportion of left crossovers than in germinal crossing over and also considerably lower than in the experiment with y sn^3. That no case of double crossing over was encountered agrees with the rarity of left crossovers. A nearly identical result was obtained in experiment 2 which was of a similar nature as 1 except for the use of f^5 instead of sn^3. In spite of f^5 being 35.7 map units farther away from the left end than sn^3 the proportion of left to right crossovers was still 8:168, disregarding the two twin spots in the last column. Again no double crossover types occurred. (One of the two twin spots covered the whole left mesothorax except the scutellum with a division line posteriorly from the anterior notopleural, presutural and anterior dorsocentral bristles which separated the anterior $+^f$ part from the posterior f^5 part; the other not quite decisive spot was formed by a left $+^f$ ant. postalar and f^5 ant. scutellar bristle. These spots can formally be explained by two crossover processes, first one to the left of f^5, resulting in a $+/f^5$ cell, and a second one occurring in this cell or one of its near descendants and segregating +/+ and f^5/f^5 twins.)

Summing up experiments 1 and 2, it is seen that the relative frequency of left crossovers is very low. Especially interesting in view of the occurrence of 17 cases of left crossovers with normal disjunction of the fibre points is the complete absence of left crossover cases with non-disjunction of the fibre points. As pointed out before, they would have been visible

TABLE 10*

Spots in flies containing $y\ sn^3$ and Mn.

In exp. (2) some of the individuals were of the constitution XXY (see p. 709).

In exp. (3) the not-Mn X chromosome carried besides y, the genes g^2 and ty (tiny bristles). The presence of the latter generally made a separation of Mn and $+^{Mn}$ spots impossible

EXP.	CONST.	IND.	SPOTS	% SPOTS	+			$y\ sn^3$			y			sn^3			y-sn^3		+ ♂ COLOR
					1	2	>2	1	2	>2	1	2	>2	1	2	>2	2	>2	
(1)	$\frac{+\ sn^3\ Mn}{+\ +\ +}$	1038	29	2.8	5	2	8	3	—	—	3	2	1	3	—	—	—	—	2
	Totals				**15**			**3**			**6**			**3**					**2**
(2)	$\frac{y\ +\ Mn}{+\ sn^3\ +}$	349	254	72.8	3	—	1	—	—	—	33	6	1	60	23	112	3	4	8
	Totals				**4**						**40**			**195**			**7**		**8**
(3)	$\frac{+\ sn^3\ Mn}{y\ +\ +}$	1040	121	11 6	—	—	—	—	—	—	25	9	69	11	1	3	1	2	—
	Totals										**103**			**15**			**3**		
(4)	$\frac{+\ +\ Mn}{y\ sn^3\ +}$	827	96	11.6	—	—	—	25	13	51	4	1	—	2	—	—	—	—	—
	Totals							**90**			**5**			**2**					

* In exp. (1), the 3 $y\ sn^3$ spots and 2 of the sn^3 spots were probably *Mn*.

In exp. (2), 4 *y* spots were probably *Mn*, 103 sn^3 spots were $+^M$, and one sn^3 spot had very small setae.

In exp. (3), 10 sn^3 spots were probably *Mn*.

In exp. (4), 33 $y\ sn^3$ spots were $+^M$, one was probably *Mn*. The total of 90 $y\ sn^3$ spots includes one of unrecorded area. Of the *y* and the sn^3 spots one had small setae and one setae similar to $+^M$.

spots with Bld sn^3 (or Bld f^5) setae. Their absence shows that non-disjunction of the daughter fibre at least in these cases of left crossing over either does not occur at all or is rare.

In experiments 3 and 4 only one type of spot was found. This type corresponds in origin to most of the spots in 1 and 2 namely either to the single crossovers to the right or to the left crossovers, both with normal disjunction of the daughter fibre points. Each of these types gives rise to ++ spots. Bld sn^3 (and ++) spots would have been produced if single crossing over and non-disjunction of daughter fibres or if double crossovers had occurred. The absence of such spots agrees with the absence of corresponding spots in 1 and 2.

Finally, it is easily seen that no chromatid reduction has taken place, as certain types of twin spots should have occurred in all four experiments with Blond. Their absence is proof of segregation, two strands by two strands, without reduction.

Experiments involving *y*, sn^3, and *Mn*

The finding of somatic crossing over along the length of the X chromosome seems to contradict BRIDGES' (1925) results with spots in Minute-n flies. He states that no spots occurred which showed recessive characters determined by genes located in the *Mn*-carrying chromosome nor that spots occurred which showed only some, but not all, characters determined by genes in the other X. In the language of the crossing over theory this would mean no somatic crossing over occurred along the main length of the chromosomes, from *y* (0.0) to *Mn* (62.7). In order to clear up the apparent discrepancy, flies heterozygous for *y*, sn^3, and *Mn* were produced and scrutinized for spots. All four possible different distributions of these three factors and their alleles over the two X chromosomes were investigated. The results are assembled in table 10. In most spots no distinction could be made between *M* and $+^M$ seta length. Wherever this character could be classified it is recorded in the footnote. It is apparent that in each of the four experiments more than a single type of spot was found. However, in each case one type predominates. In the only case in which this prevalence amounts to less than 75 percent of all spots an external cause can be seen to be responsible, namely the most frequent class of spots in experiment 1 is distinguished from the surrounding tissue by its $+^M$ condition only. Spots of this constitution are recognizable, in general, only if they include one or more macrochaetae on the head or thorax, while all other spots exhibiting *y* or sn^3 or both *y* and sn^3 are recognizable anywhere and both in macro- and microchaetae. If only those spots are tabulated which have about equal chances of being recognized, namely head-thorax spots which include macrochaetae, their numbers are found to be

TABLE 11

*Results of **x** (equational) and **y** (reductional) segregation in experiments involving y, sn^3, and Mn. In each case the upper symbols represent one segregate and the lower symbols represent its twin segregate.*

EXP.	SINGLE CROSSOVER IN REGION	SEGREGATION*	
		(x)	(y)
(1)	1	$y\ Mn$ Mn	dies $+$
$\frac{y\ sn^3\ Mn}{+\ +\ +}$	2	$y\ sn^3\ Mn$ Mn	dies $+$
	3	dies $+$	Mn Mn
(2)	1	$y\ Mn$ Mn	dies sn^3
$\frac{y\ +\ Mn}{+\ sn^3\ +}$	2	$y\ Mn$ $sn^3\ Mn$	dies $+$
		dies sn^3	Mn Mn
(3)	1	Mn $y\ Mn$	dies $+$
$\frac{+\ sn^3\ Mn}{y\ +\ +}$	2	$sn^3\ Mn$ $y\ Mn$	dies $+$
	3	dies y	Mn Mn
(4)	1	Mn $y\ Mn$	dies sn^3
$\frac{+\ +\ Mn}{y\ sn^3\ +}$	2	Mn $y\ sn^3\ Mn$	dies $+$
	3	dies $y\ sn^3$	Mn Mn

* **z**-segregation yields always: $\begin{matrix}Mn\\Mn\end{matrix}$

14+ and 2*sn*^{3}, thus making experiment 1 fit in with the others as to the striking prevalence of one type of spot. In order to find out the processes by which the different types of spots were produced, table 11 was constructed; it gives the types of spots to be expected in case of single somatic crossing over in the regions (1) *y*-*sn*^{3}, (2) *sn*^{3}-*Mn* and (3) *Mn*-spindle fibre point and with **(x)** equational or **(y)** reductional segregation of the, daughter fibres. Another possible equational separation, **(z),** will not lead to visibly different spots. It is assumed that no chromatid reduction takes place. The contrary assumption can be disproved as in previous discussions.

An analysis has to be based on a consideration of *all* four experiments. Regarding the + spots in experiment 1 it is seen that three different processes of crossing over and segregation, namely **(1y), (2y),** and **(3x)** lead to + spots. As we expect the same processes in comparable frequencies to occur in experiments 2 to 4, we have to find out from table 11 what kinds of spots would result from these processes.

(a) Process **1y** would lead to *sn*^{3} in experiment 2, + in experiment 3, and *sn*^{3} in experiment 4. Indeed, experiment 2 yielded *sn*^{3} spots as the largest class, in conformity with the + spots constituting the largest class in experiment 1. In experiment 3, however, no + spots were found and in experiment 4 the *sn*^{3} spots constituted the smallest class. Thus process **1y** does not fulfill the requirements.

(b) Process **2y** can be excluded also. It would lead to + spots in all four experiments, while + spots actually form the smallest class in experiment 2 and do not occur at all in experiments 3 and 4.

(c) The remaining process **(3x)** would give results in accordance with all actual findings, as the different kinds of spots to be expected from it represent indeed the most frequent class in each experiment.

A similar analysis, carried out in regard to the less frequent classes of spots leads to the following results: Process **1x,** crossing over between *y* and *sn*^{3} and equational fibre point segregation can account for *y* spots in experiments 1 to 4; process **2x,** crossing over between *sn*^{3} and *Mn* with equational fibre point segregation can account for *y sn*^{3} spots in experiments 1 and 4 and for *y*-*sn*^{3} twin spots in experiments 2 and 3. It is seen in some cases that certain classes of spots are made up from products of different processes, as in the case of *y* spots in experiment 3, where the majority is considered to have originated in consequence of **3x,** while some must be products of **1x.** Furthermore, whenever *y*-*sn*^{3} twin spots are expected, as in experiments 2 and 3, some of these will cover only so small an area as to appear as single *y* or *sn*^{3} spots and will therefore add to the heterogeneity of the *y* or *sn*^{3} classes. In some of these cases the different origin should lead to spots which contain either Minute-n or its normal allele. As indicated in table 10, both types of spots were frequently found in the different classes and were present in proportions which were roughly in conformity with expectations.

There are a few types of spots which cannot be accounted for on the basis of single crossovers namely in experiment 1, 3 sn^3 spots, 2+ ♂ colored spots; in experiment 2, 4 + spots, 8+ ♂ colored spots; in experiment 4, 2 sn^3 spots.

A discussion of the + ♂ colored spots will be postponed (cf. p. 667.) The remaining few spots can be accounted for by double (and triple) crossing over, as can be readily seen. The occurrence of such multiple crossovers should lead to certain other spots also which would not be distinguishable from the spots produced by the single crossover processes. This adds slightly to the heterogeneity of the main classes.

Table 12

Number of spots with 1 or 2 and with more than 2 setae in experiments 1-4 from table 10

EXP.	PREVALENT CLASS		ALL OTHERS	
	1-2 SETAE	>2 SETAE	1-2 SETAE	>2 SETAE
(1)	7	8	11	1
(2)	83	112	45	6
(3)	33	69	13	5
(4)	38	51	7	0
	161	240	76	12
Totals	**401**		**88**	
% of spots >2	60		14	

The following summary of the results of these tests is based on the as yet unproved assumption that apparent non-crossovers are really crossovers between the rightmost locus investigated (*Mn*) and the fibre point. We then find: (1) the most frequent cause of spots is somatic crossing over in the region nearest the fibre point, region 3, and equational segregation of the fibre points; (2) somatic crossing over in other regions of the X chromosomes occurs also and if followed by equational divisions of the fibre points accounts for the less frequent types of spots.

The relative frequencies of the different types of crossovers will be considered after one more result has been pointed out.

If we tabulate (table 12) for experiments 1 to 4 the numbers of spots which cover only 1 or 2 setae and those which cover more than 2 setae, we see that 60 per cent of all spots in the prevalent class and only 14 per cent of all other classes belong to the larger kind. The difference becomes more significant still if we remember that part of the spots in the main class belong in reality to the other group and thus should tend to increase the number of small spots in the prevalent class. This striking correlation between chromosome region of somatic crossing over and size of spot might be accounted for on two different assumptions (other possible but less probable causes will not be discussed): (a) The products of crossing over to the left of *Mn* might have lower viability than those of crossing over to the right so that their growth is more restricted; (b) the relative frequency of crossing over to the left of *Mn* increases with the age of the developing insect, so that late cell divisions are more frequently preceeded by such kind of crossing over. As the size of the spot area formed decreases the later the time at which segregation takes place, crossovers to the left of *Mn* would result in a relatively high number of small spots.

Assumption (a) can be rejected, as there is no obvious reason why viability differences should differentiate between crossovers to the left or to the right of *Mn*, especially as certain spot constitutions which represent left crossovers in one experiment signify right crossovers in another. This leaves assumption (b) as an explanation of the average size differences of the different types of spots. *In Mn flies the region of somatic crossing over along the X-chromosome is correlated with the developmental stage of the organism.* No such relation was found in the not-*Mn* flies recorded in table 8.

A calculation of the relative frequencies of crossing over in different regions is made very difficult by this finding. It is probable that many cases of segregation after crossing over to the left of *Mn* occur so late that no seta is involved and the mosaic area remains undetected. The relative numbers of observed spots belonging (primarily) to the right and to the left crossover group is therefore biased to the disadvantage of the smaller left crossover spots. How great this bias is can hardly be estimated at present, but it seems very doubtful that it will be great enough to establish a ratio between somatic crossovers to the left and to the right of *Mn* which equals the ratio in germinal crossing over. If equality existed, the chance of observing a left crossover spot would have to be only 1/95 of that of observing a right crossover one, as the germinal ratio is of the order of 19:1 and the observed somatic ratio is of the order of 1:5 (88:401). If this chance is considered too small, it follows that somatic crossing over should be more frequent in the immediate neighborhood of the spindle fibre than in other regions of the X chromosomes. This finding agrees with the similar though less pronounced concentration of crossovers to the right of sn^3 observed in the experiments described in table 9 and table 8. However, such a calculation based on the *total* of observed spots obscures the fact that at different stages of development the relative frequencies of somatic crossovers in different chromosome regions vary.

The correlation between region of crossing over and developmental stage throws light on the divergence of our findings of more than one type of spot and the earlier findings of BRIDGES in which only one kind of spot, explicable by crossing over to the right of *Mn*, had been observed. BRIDGES focussed his attention on the larger spots, not on single seta spots; and he restricted his observation mainly to the head and thorax. As the great majority of spots originating after crossing over to the left of *Mn* comprises only 1 or 2 setae, it is probable that they have been overlooked formerly. Only 12 out of 489 spots summarized in table 12 covered more than 2 setae and only 3 of these occurred on head or thorax. It is not surprising that such a small number of aberrant spots should have gone unobserved.

There is one more point of divergence between the earlier results and interpretations of spots in *Mn* flies and the present ones. It relates to the number of X chromosomes present in cells of the mosaic area. This question will be considered separately.

Experiments involving *y*, *sn*^{3} and "Theta"

No proof has been given as yet for the statement that the apparent noncrossover spots were indeed results of crossingover to the right of the rightmost locus considered. This will be demonstrated now and it will be shown that somatic crossing over is a general process preceeding somatic segregation.

As indicated in the introduction, an experiment was undertaken to determine whether the whole chromosome was lost during the supposed elimination of the *Mn* chromosome. All former findings except those described in the last section had pointed to an "elimination" of at least most of the *Mn* X chromosome—from yellow (0.0) to the right of *Mn* (56.7). There were no "good" loci known to the right of *Mn* which could be tested with respect to their possible elimination. However, MULLER'S finding of an induced, cytologically visible duplication, Theta (θ), had provided a tool for this investigation (PATTERSON 1930). Theta is a deleted X chromosome comprising the left end with the loci yellow, scute, and broad (0.0–0.6) and bobbed from the right end. In the stocks used Theta was attached to the right end of a normal X. The chief property by which the presence of Theta is ascertained is its possession of a normal allele of yellow. In males or females in which the normal X chromosomes contain the recessive mutant yellow, the presence of Theta can be seen by their not-yellow normal body color.

When females of the constitution *y Mn* θ/*y* were inspected for spots of not-Minute setae, the color of these spots was expected to be yellow in case a complete elimination of the *y Mn* θ chromosome had taken place and to be normal when only the *y Mn* part, but not the θ part of the chromosome had been lost. In a first experiment of this type 26 spots were found all not-yellow. This seemed to prove that only a part of the X was eliminated and that a break had occurred between *Mn* and the spindle fibre insertion, where Theta is attached. When, however, females of a similar constitution were examined in which the Theta-fragment was attached to the not-*Mn* chromosome ($y\ Mn/y\ \theta$), it was discovered that the not-*Mn* spots were of two kinds, some being not-yellow, others being yellow. The first kind would be expected from a simple elimination of most or all of the *y Mn* chromosome. But the yellow spots seemed to show that *together with the Mn-containing part of one X chromosome the Theta part of the other X chromosome had disappeared.* A full analysis of these findings will be given in a later chapter. Here we shall follow the implications in regard to mosaics in which no sex-linked Minute is involved.

When somatic segregation had been recognized as the process leading to mosaic formation it was obvious that an interpretation of the Minute-Theta results involved assumptions of somatic crossing over to the right of *Mn*. Accordingly Theta was introduced into females of constitution otherwise similar to that described in the preceding chapter (the presence of *Hw* and dl-49 may be disregarded, but see p. 708). Table 13 contains the results. Two main types of females were inspected: (1) containing *y* and sn^3 in one X and *y* and Theta in the other and (2) containing *y*, sn^3 and Theta together in one X and only *y* in the other one. In these experiments due to the homozygous condition for *y* at the left end of the X chromosomes, crossing over between the X chromosomes, if it occurred at all, could be observed in its effect only when the exchange had taken place between the locus of sn^3 and the attachment point of Theta. Three main possibilities seemed to exist: (a) no somatic crossing over, (b) somatic crossing over occurring at a two strand stage with respect to the two X chromosomes, each chromosome being still represented as a single strand, (c) somatic crossing over at a four strand stage. These might be regarded to be of unequal probability if considered in the light of our foregoing analysis and of cytological results. But the analysis offered a new test of the findings reported above and it was also thought best to judge the genetic evidence of these experiments on its own merit as much as possible. This will be done herewith.

(a) No somatic crossing over. In this case somatic segregation in experiment 1 should have yielded two daughter cells with the constitutions $y\ sn^3$ and $y\ \theta$ giving rise to $y\ sn^3$ spots ($y\ \theta$ spots are phenotypically indistinguishable from the surrounding tissue). Only two $y\ sn^3$ spots out of 143 spots were found, showing that segregation without crossing over cannot account for the findings.

TABLE 13

Spots in experiments involving primarily y, sn^3 and θ. Part of the individuals contained an autosomal Minute in order to increase the frequency of spots.

EXP.	CONSTITUTION	INDIVIDUALS	SPOTS	sn^3			y			y sn^3		y-sn^3		y sn^3-sn^3	+♂ COL.
				1	2	>2	1	2	>2	1	>2	2	>2	>2	
1a	*y sn^3/y θ*	170	53	16	5	20	3	2	—	1	1	—	2	1	2
b	*y sn^3 bb/y Hw* dl49 *θ*	268	59	33	10	11	4	—	1	—	—	—	—	—	—
c	*y sn^3 bb/y θ*	2558	31*	13	1	7	8	—	—	—	—	1	1	—	—
				62	16	38	15	2	1	1	1	1	3		
Totals Exp. 1		**2996**	**143**	**116**			**18**			**2**		**4**		**1**	**2**
2a	*y sn^3 θ/y Hw* dl49	679+	148	87	28	21	6	2	3	—	—	1(?)†	—	—	—
b	*y sn^3 θ/y w*	338	39	22	6	1	8	—	1	—	—	—	1	—	—
				109	34	22	14	2	4	—	—	1(?)	1		
Totals Exp. 2		**1017+**	**187**	**165**			**20**			—		**2(?)**		—	—

* In exp. 1c only head and thorax were inspected. All 31 spots were found on the thorax.

† Doubtful if the single bent seta was genetically sn^3.

The same conclusion holds true for experiment 2. Here simple segregation should produce two daughter cells of the constitutions *y sn*^{3} θ and *y*, giving rise to *sn*^{3} and *y* twin spots. But only two twin spots (one doubtful!) out of 187 were found.

(b) Somatic crossing over at a two strand stage.

Expectation in experiment 1: two daughter cells with the crossover constitutions *y sn*^{3} and *y*; these should result phenotypically in *sn*^{3} and *y* twin spots.

Result: only 2 such twin spots out of 143 spots.

Expectation in experiment 2: two daughter cells with the crossover constitutions *y sn*^{3} and *y* θ giving rise to *y sn*^{3} spots (the *y* θ area not being distinguishable from the surrounding tissue).

Result: No *y sn*^{3} spot out of 187 spots.

Conclusion: Segregation with crossing over at the two strand stage does not account for the findings.

(c) Somatic crossing over at a four strand stage. This implies for our discussion that only two strands at any one level cross over. Otherwise four strand crossing over would here be indistinguishable from two strand crossing over. Before entering a detailed analysis, it will be shown once more that generally no chromatid reduction occurs in the formation of mosaic areas.

We consider the original constitution of the females in experiment 1 and experiment 2. If reduction occurs in experiment 1 two of the four resulting cells would each obtain a non-crossover strand of the constitution *y sn*^{3}, or *y* θ, and the other two cells would each obtain a crossover strand of the constitution *y sn*^{3} θ or *y*. The areas resulting from later divisions of the four reduction products would exhibit the phenotypes *y sn*^{3}, +, *sn*^{3}, and *y*. As the phenotype + is not different from the surrounding not-reduced tissue, the visible result would be a triplet spot with a *y sn*^{3}, *sn*^{3}, and *y* area. No spot of this nature was observed and the *y sn*^{3} phenotype itself appeared not more than three times in 143 spots. As to experiment 2: the four reduction products would have the constitutions *y sn*^{3} θ, *y*, *y sn*^{3}, and *y* θ, giving rise to a triplet area with the phenotypes *sn*^{3}, *y*, and *y sn*^{3} as in experiment 1. Again no such spot was found nor a single *y sn*^{3} phenotype out of 187 spots.

Thus there remains the following mechanism to be examined: crossing over between two of the four strands and subsequent segregation two by two of strands without chromatid reduction. The main consequences of this mechanism are diagrammatically represented in figures 8 and 9. At the left of the first horizontal section (a) the four chromatids are represented which originated as a consequence of crossing over in the *sn*^{3}-Theta-attachment region between two of the original chromatids. To the right of this

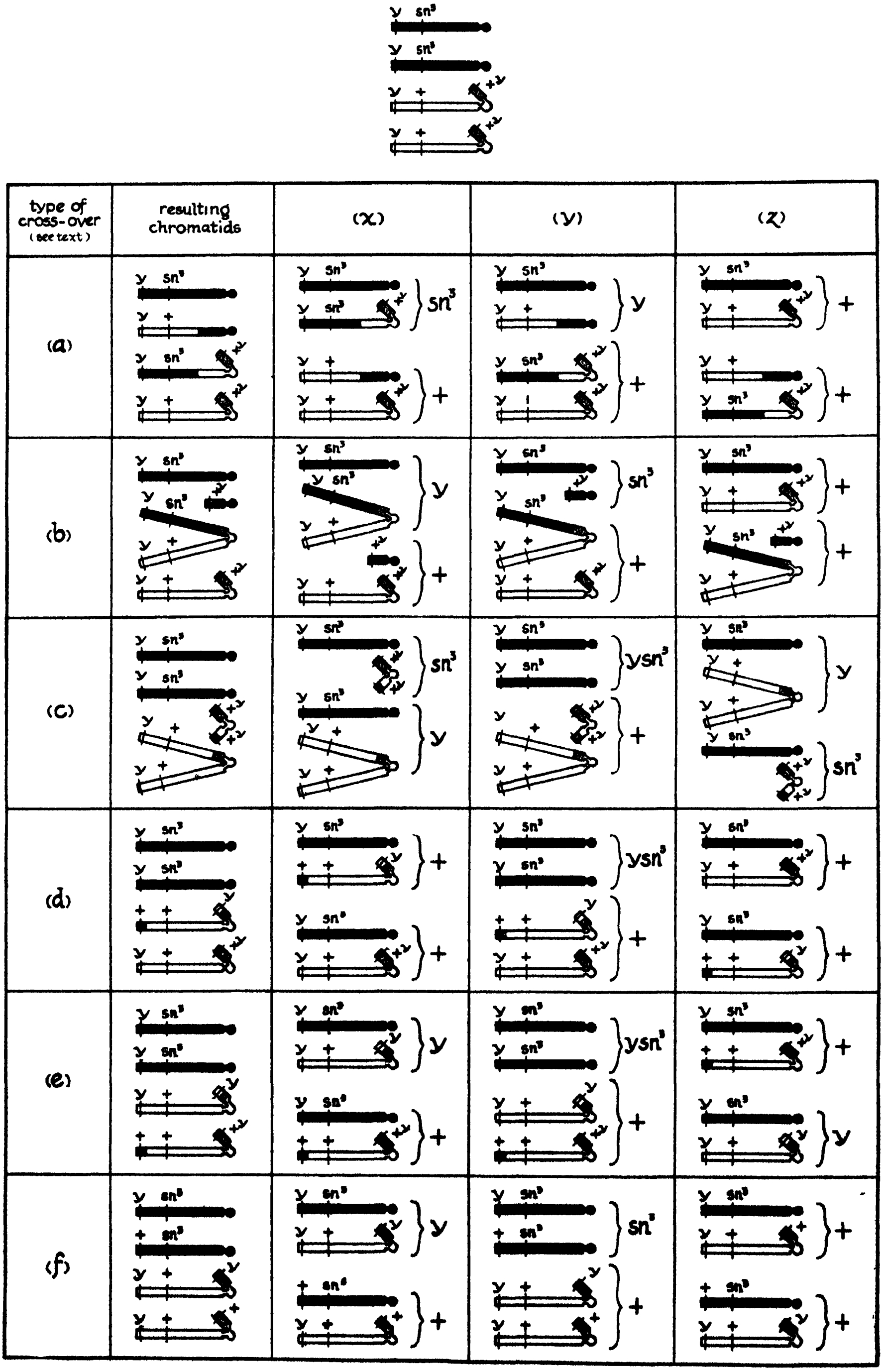

FIGURE 8. $y\ sn^3/y\theta$. (See table 13, experiment 1.) Six types of single crossovers and the results of three different types of segregation.

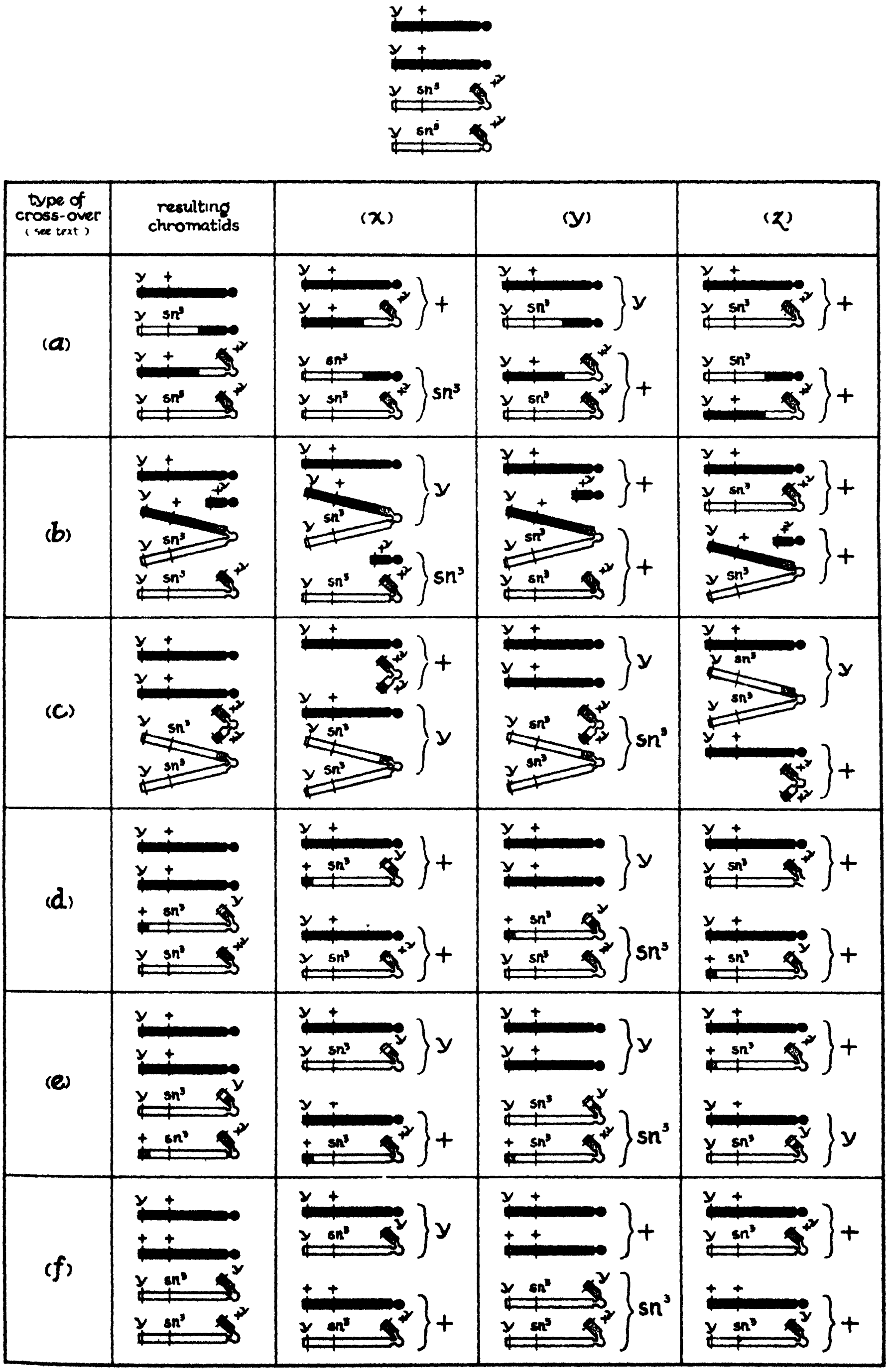

FIGURE 9. $y/y\ sn^3\theta$. (See table 13, experiment 2.) Six types of single crossovers and the results of three different types of segregation.

four chromatid group are shown the results of the three different possibilities of distributing the chromatids two by two into two daughter cells. In both experiments the first of these possible types, **x**, leads to the appearance of a *sn*^{3} spot, the second, **y**, to that of a *y* spot while the last, **z**, does not give rise in any of the two resulting cells to a phenotype different from the surrounding tissue. If we compare these deductions with the actual results (table 13) it appears that the great majority of spots seem to fit the expectation. In experiment 1, 134 spots out of 143 and in experiment 2, 185 out of 187 spots exhibit phenotypes which would result from the mechanism discussed. The proportion of *sn*^{3} to *y* spots in both experiments is similar also, namely 116:18 in experiment 1, and 165:20 in experiment 2. Before accepting the conclusion that crossing over to the left of the Theta-attachment and an ensuing chromatid distribution according to two different types in a proportion of about 8:1 is responsible for the findings, it will be necessary to see whether there are other types of crossing over and segregation which would lead to the same observed spots. Five further types of crossing over are listed in figures 8 and 9. In the horizontal sections (b) and (c) the possibility is considered that crossing over involves the right (bobbed) end of the Theta fragment and that part homologous to it which is located near the spindle fibre in the X chromosome—(b) in the free X and (c) in the X chromosome to which Theta is attached. In the sections (d)-(f) the possibility is considered that crossing over involves the left (yellow) end of the Theta fragment and the homologous part in the left end of the X chromosomes—(d)between a Theta chromatid and the chromatid of the X chromosome to which the Theta chromatid is attached, (e) between a Theta chromatid and the sister X chromatid of that to which the Theta chromatid is attached, and (f) between a Theta chromatid and a chromatid of the free X chromosome. If somatic crossing over obeys the same rule as does germinal crossing over, then it can be predicted that the crossover processes represented in sections (b)–(f) will play only a very small role, if any, in comparison to the crossover type referred to in (a). This follows from the generally established fact that short duplications take very little part in germinal crossing over (DOBZHANSKY 1934). In addition there may be quoted the results of unpublished experiments on germinal crossing over in the X chromosomes of females possessing one Theta attachment: Only 2 out of 7559 individuals occurred as a result of crossing over between Theta and the homologous region near the spindle fibre of an X chromosome. But in spite of the rareness of such crossing over types during germ cell formation, we have to consider the consequences of their possible occurrence in somatic tissues if followed by segregation. For, lacking any previous acquaintance with somatic crossing over, we are not justified at this stage to apply our knowledge of germinal processes to somatic ones.

The procedure will be to tabulate which types of crossing over and segregation are able to give rise to the two main kinds of spots which were observed, namely sn^3 and *y*.

It is seen (fig. 8) that sn^3 spots originate in experiment 1 only according to the types **ax, by,** and **fy.** In experiment 2 (fig. 9) sn^3 spots can originate according to types **ax** and **fy.** Other types in which sn^3 appears as part of a twin segregate will not be considered for the moment.

The process **by** in experiment 2 does not give rise to sn^3 spots. It therefore seems sufficient to consider only **ax** and **fy** as possibly responsible for the observed sn^3 spots. Now the process **fy** should hardly occur without the occurrence of **dy** and **ey,** for a striking preference of the Theta fragment for crossing over with different kinds of the distant yellow ends of X chromosomes is not likely. However, **dy** and **ey** give rise to *y* sn^3 spots in experiment 1 and to *y* and sn^3 twin spots in experiment 2. Such spots were negligible in number—and they can also originate through different processes. It is therefore concluded that no appreciable percentage of **fy** processes occurred and that the majority of *the* sn^3 *spots both in experiment 1 and 2 were produced as results of the process* **ax.**

Yellow spots would appear as a result of the following processes in experiment 1: **ay, bx, ex, ez,** and **fx.** In experiment 2 yellow spots will result from **ay, cx, cz, ex, ez,** and **fx.** The processes **ey, ex, ez,** and **fx** agree in both experiments in yielding only *y* spots. On the other hand, **bx** would be responsible for *y* and sn^3 twin spots in experiment 2, and **cx** for *y* and sn^3 twin spots in experiment 1. But twin spots have to be considered also, as some of them will appear phenotypically only as *y* areas (others as sn^3 areas). Besides **bx, cx,** and **cz** we have, therefore, to discuss still the processes **cy, by,** and **ey,** which give rise to *y* and sn^3 twin spots in experiment 2. The last named group of processes, if they occur, gives rise to *y* sn^3 spots in experiment 1. Actually only 2 *y* sn^3 spots out of 143 were found in experiment 1 so that these processes at best can account only for a small number of *y* spots in experiment 2 and cannot account at all for any of the 18 *y* spots in experiment 1. We are then restricted to an analysis of the types **ay, bx, cx, cz, ex, ez,** and **fx.** Do all these crossing over and segregation processes occur? A decision cannot be reached without taking recourse to other data. We have seen in the preceding chapter on spots in *Mn* or Bld-bearing females that the occurrence of types of segregation which are reductional with respect to sister fibre attachment points could be excluded. If we use this knowledge for our present problem we see that the process **ay** representing such a reductional segregation becomes eliminated and only equational segregation processes remain. They all have in common the fact that they involve crossing over between the Theta duplication and the X chromosome, **bx, cx** and **cz** crossing over in the homologous right regions and **ex, ez** and **fx** in the homologous left regions. Whether both kinds of crossovers contribute to the *y* spots or if only one kind occurs and if so, which one it is, cannot be ascertained from the present experiments. But further information will be yielded in an analysis of experiments which involve *y*, sn^3, *Mn*, and θ. No discussion will be devoted to the *y* sn^3 spots nor to the sn^3 and *y* sn^3 twin spot. Simple elimination, non-disjunction processes, or multiple crossovers at one or successive stages may be involved.

The results of this chapter can be summarized as follows:

(1) Somatic segregation is always (or nearly always?) preceded by somatic crossing over between two of four chromatids.

(2) If the result of a preceding section is held to be generally true, namely, that segregation is always equational with respect to spindle fibre attachments, then it is found that somatic crossing over involves not infrequently the Theta-duplication which only very rarely participates in germinal crossing over.

On the basis of these findings it is postulated that somatic crossing over underlies segregation also in those cases where the genetic constitution, on account of the absence of an attached Theta duplication, does not seem to allow a direct test of crossing over in the rightmost region. Such a test, however, is possible under some circumstances as will be shown in the following section.

Experiments involving bobbed as a means of determining the rightmost crossover region

As pointed out before, the fact had been unexpected that in flies heterozygous for bobbed (*bb*) or one of its alleles "elimination" of the not-bobbed X chromosome generally does not result in spots exhibiting the bobbed phenotype. Table 14, experiments 2 and 3, contains data on such spots in flies which had *Mn* in one X and *bb* or bb^l (a lethal allele of *bb*) in the other one. It is seen that 34 of the spots in experiment 2 possessed phenotypically non-*bb* setae, and that in experiment *3* where only non-*bb* spots would have been visible, 10 spots were found. Leaving aside at present the spots with smaller than normal setae, the non-appearance of the *bb* character in these spots has to be explained.

The assumption of non-autonomous development of the bobbed character from cells of a mosaic area, although possible, seemed unlikely, especially in view of the result of an extensive test of the sex-linked factor "tiny bristle" (*ty*, 1, 44.5) which is very similar in action to bobbed, to-

TABLE 14

Seta length in spots of individuals containing bb and bb^l (exp. 2 and 3). Experiment 1 is the control.

EXP.	CONSTITUTION	INDIVIDUALS	SPOTS	TYPE OF SPOT (LENGTH OF SETAE) +	<+
1	$\frac{+\ g^2\ Mn}{y\ +\ +}$	528	58	54	4†
2	$\frac{+\ g^2\ Mn\ +}{y\ +\ +\ bb}$	769	45	34	11*
3	$\frac{+\ g^2\ Mn\ +}{w^e\ +\ +\ bb^l}$	476	10	10	?

† Only spots are recorded which enclose macrochaetae on head or thorax They were *y* in exp. 1 and 2 and $+^M$ in exp 3 In the latter experiment spots with setae shorter than $+^M$ could not be distinguished from the rest of the body

‡ Setae length as in *bb* ♀ : 2 spots
Setae length >"*bb* ♀," but <+: 2 spots

* Setae length as in *bb* ♀ : 4 spots
Setae length >"*bb* ♀" but <+: 6 spots
Setae length like *bbXO*♂: 1

gether with which it can be classified as a recessive Minute. The test consisted in observing whether *ty* behaved autonomously in spots or not. The result was that it did, which made the discordant behavior of *bb* more surprising.

The theory of somatic crossing over in the form proposed allows us to understand the apparently exceptional behavior of *bb* if we make a specifying assumption concerning the region of somatic crossing over in the X. It has been pointed out before that in the majority of cases this region lies between the locus of *Mn* and the right end of the X. If the point of crossing over were located between the rightmost factor, which is bobbed, and the right end of the X, then the behavior of the phenotype bobbed in spots would be exceptional also under the somatic crossing over theory, for the equational segregation of fibre attachment points is by necessity reductional for all loci to the left of the crossover point. Therefore one of the segregation products of a fly which is heterozygous for bobbed becomes homozygous for this locus. Thus in a *Mn/y bb* female crossing over at the four strand stage, if occurring to the right of *bb*, would lead to daughter cells of the constitutions *Mn/Mn* and *y bb/y bb*, the latter giving rise to *y* spots. These spots, in spite of their assumed homozygous condition for *bb*, show long not-*bb* bristles. Or let us consider experiment 4 of table 5. In females of the constitution *y* . . . bb^l/sn^3bb^x, four strand crossing over to the right of the *bb* alleles should yield daughter cells of the constitutions $y \ldots bb^l/y \ldots bb^l$ and sn^3bb^x/sn^3bb^x. From the knowledge of the recessive lethal action of bb^l in zygotes the most likely assumption would be a cell lethal action which hinders the $y \ldots bb^l/y \ldots bb^l$ cell from developing into a spot area. However, two *y* areas in y-sn^3 twins were found covering 3 setae and four covering 2 setae besides numerous one-seta *y* areas (tables 7 and 5). The difficulties disappear if we assume that the point of somatic crossing over lies to the left of the *bb* locus. This assumption agrees with the fact that as a rule no *germinal* crossing over occurs between *bb* and the fibre locus (STERN 1929). Crossing over to the left of *bb* will leave the constitution of the daughter cells unchanged with regard to *bb*. In *Mn/y bb* flies the daughter cells will be *Mn* +/*Mn bb* and *y* +/*y bb* and the resulting *y* spot, heterozygous for *bb* like the rest of the fly, will have not-*bb* bristles; in $y \ldots bb^l/sn^3bb^x$ females the twin spots will have the constitutions $y \ldots bb^l/y \ldots bb^x$ and sn^3bb^l/sn^3bb^x, again not differing from the surrounding tissue in regard to *bb*.

There remains to be given an explanation of the 11 spots in experiment 2 which exhibited *y* setae of shorter than normal length. That they are not due to a special behavior in regard to *bb* is probable from the control experiment 1 in which *bb* was not involved at all and in which 4 spots with shorter setae were found. These 4 spots are to be expected as results of crossovers between *y* and *Mn* thus producing spots of the constitution *y Mn/y* +, the variation in setae length representing the known variability of *Mn* setae in the presence of different modifiers (which in these spots must be of varying combinations due to different crossovers). The same explanation is adopted for 10 of the 11 spots with smaller than normal setae in experiment 2, while the single bristle spot with a very short, *bb* XO-like seta was perhaps due to a real elimination of the *Mn* X chromosome. The higher frequency of the smaller spots in 2 than in 1 is within the limits of variability of somatic crossing over (the difference also being smaller than three times the standard error). It might be added that experiments 2 and 3 were conducted in such a way as to exclude the presence of a supernumerary Y chromosome which would have covered the *bb* effect.

The assumption that the crossover point lies always to the left of *bb*, can be applied to an independent test of the origin of the *y* spots which appear in the Theta experiments discussed in the preceding section. We had seen that the *y* spots may have resulted both from a reductional segregation process after crossing over between the X chromosomes (**ay** process) and from equational segregation processes after crossing over involving the Theta duplication (processes **bx . . . fx, cz, ez**), The first alternative could not be rejected on the basis of an analysis of the pertinent data but only on the basis of evidence from earlier experiments.

If the *y* spots occurred in consequence of processes **ay,** after reductional distribution of the fibre points, then a reductional distribution for the *bb* locus would have occurred also. Bobbed then should exhibit its phenotype in suitable spots. But if the *y* spots were due to any of the processes **bx . . . fx, cz, ez,** then *bb* should not appear phenotypically. This point could have been tested in experiments 1b and 1c of table 13. In experiment 1b five *y* spots occurred. A reliable distinction of *bb* and not-*bb* setae generally can be made only in macrochaeta of the head and thorax and in flies which do not carry a Minute factor. Only 3 of the 5 *y* spots occurred in non-Minute flies and none included head or thorax macrochaetae. Experiment 1c represents a special attempt to obtain *y* spots covering suitable setae. Of the nearly 3000 individuals inspected only 18 possessed *y* head-thorax spots, 4 of which covered macrochaetae. Three of these spots had setae which were clearly of about normal, not bobbed size; one had setae of a size similar to *bb*. This last named *y* spot seems to indicate the occurrence of the **ay** process. However, too much weight should not be laid on this single finding which is contradicted by sufficient other evidence. It could, for instance, be regarded as a case of crossing over to the right of *bb* with normal disjunction of the fibre points. However, this is not suggested as a probable explanation.

The other three not-bobbed, yellow spots establish definitely the occurrence of at least some of the crossing over and segregation processes **bx . . . fx, cz, ez.** It is probable that one or both of the processes **b** and **c** were involved, as the bristle size in these spots, though not-bobbed, was apparently somewhat abnormal. This points to a 3X (superfemale) constitution of the spots, as to be expected from **bx** and **cx** or **cz** (fig. 8).

The number of X chromosomes in cells of spots

If mosaic spots in females which do not contain a duplication originate by segregation of four chromatids two by two following crossing over between two X chromosome chromatids, then the X chromosomes in cells constituting the mosaic regions must be present in the diploid number This expectation is in contrast to that derived from a simple elimination hypothesis which leads to the expectation of finding one X chromosome only. Still other numbers of chromosomes can be predicted after segregation in females carrying a duplication as can be seen in figures 8 and 9. An investigation of the number of X chromosomes in mosaic spots should provide new tests for the proposed theories.

It was not thought possible to determine the number of X chromosomes by a cytological study as no cell divisions are expected in the hypodermal cells which constitute the spots of adult individuals. Genetic means, however, are available. BRIDGES (1925) used two criteria: the presence (♂ = X) or absence (♀ = XX) of a sex comb in case of a spot covering the sex comb region of a front leg and the shade of w^e (eosin 1, 1.5) color in eye spots of individuals which according to their genetic constitution exhibited eosin in a mosaic eye part. Eosin is known to be pink in females and pinkish yellow in males. Since the sex comb region is involved only very rarely in a spot and since the eosin test allows for reliable judgments of the eye color only in comparatively large spots, a third criterion for X or XX condition was used primarily in the present studies, namely, the coloration of the fifth and sixth abdominal segment.[1] These segments are dark in males and, in most stocks, colored only along the posterior margins and near the median line in females. This coloration is cell-autonomous as many cases ranging from smallest to largest black spots on female segments in certain experiments show. A fourth criterion of the X or XX constitution of spots can be derived from a consideration of bobbed spots as will be shown in the next section.

The determination of the sex of spots as an index of the number of X chromosomes was made in all later experiments after the importance of this condition was recognized. In the earlier experiments the determination of sex was not made while the flies were alive. However, it seems certain to me that I would have recorded the fact of male coloration in abdominal spots had it occurred, as I wrote down individual records for each spot noting the numbers of bristles involved, the abdominal segment, and often added a sketch of the spot. Whenever no comment was made in my notes (in 22 out of 147 cases) it can be regarded as highly indicative of the absence of male coloration, that is, of the presence of two X chromosomes.

Consequently all those spots found in the experiments discussed in the preceding sections of this paper in which two or more setae of the critical abdominal region were involved have been tabulated in table 15. Besides, the results of three further experiments reported elsewhere (STERN 1935b) have been added. It will be noted that the mosaics have not been classified under the alternative "female color or male color" but rather under "male color or no male color." The latter is better, since it is possible that in rare cases very narrow spots do not exhibit male coloration in spite of 1X constitution even if they cover two or more setae. The table shows that the great majority of mosaics, 132 out of 147, did not exhibit male coloration. They thus contain cells with the constitution XX, in agreement with the theory of crossing over and segregation and in contradiction to the elimination theory. Fifteen spots exhibited male coloration, an effect of one X constitution.

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[1] Owing to the fact that the apparent first segment is regarded to be originally composed of two segments, the morphologically fifth and sixth segments are the ones which appear as fourth and fifth without knowledge of the homologies.

The probable origin of most of these exceptional spots will be discussed later in connection with the influence of an extra Y chromosome on somatic crossing over. It is significant that no male color appeared in any of the 31 spots which exhibited the typical result of segregation, the twin condition. Here it is pointed out only that the male-colored spots occurred in a few very nearly related cultures in each experiment: 5 of the 6 male spots in line 1 of table 15 came from 3 related cultures of a total of 34, all 3 male spots in line 2 from one single culture of a total of 29 and both male spots in line 3 from one single culture of a total of 32 This indicates that special genetic conditions were responsible.

TABLE 15

The sexual coloration in critical abdominal spots.
Numbers in ( ) represent those of the total number of spots which were recorded without a comment as to sexual coloration.

EXPERIMENTS	MAIN FACTORS INVOLVED	sn^3		y		$y\ sn^3$		y-sn^3		+	NO ♂ COL
		NO ♂ COL	♂ COL	NO ♂ COL.	♂ COL	NO ♂ COL	♂ COL	NO ♂ COL	♂ COL	♂ COL	♂ COL
Table 5, 2-4†	y, sn^3	7(2)	3	7(3)	3	—	—	22(1)	—	—	36:6
Table 8, 1-4	y, sn^3	—	—	9	—	13	1	—	—	2	22:3
Table 10, 1–4‡	y, sn^3, Mn	—	—	2	—	7	—	—	—	2	9:2
Table 13, 1a, b	y, sn^3, θ	12(3)	—	1(1)	—	—	1	—	—	2	13:3
Table 13, 2a*	y, sn^3, θ	9(9)	—	4(3)	—	—	—	—	—		13:0
$y\ Hw/sn^3$		1	1(?)	—	—	—	—	9	—	—	10:1(?)
$y\ Hw$ dl-49′/+		—	—	16	—	—	—	—	—	—	16:0
$(y\ sc)^-/sn^3$		13	—	—	—	—	—	—	—	—	13:0
									Total·		132(22):15(?)

† No abdominal spots were recorded in exp 1
‡ No critical spots occurred in exp 3; for exp 2 see text
* No critical spots occurred in exp 2b.

The two normal, male-colored spots in line 4 will be commented upon later.

A comprehensive experiment involving y, sn^3, Mn and Theta

Each of the experiments dealt with in the preceding chapters yielded results which, taken together, furnish a consistent picture. However, some points were cleared up only by referring from one experiment to another and other points were left completely unsolved. The present chapter gives an account of experiments in which y, sn^3, Mn, and θ were involved at the same time. Emphasis will be laid on two questions: (1) Are the y spots

TABLE 16a

Spots in experiments involving primarily y, sn^3, Mn, and θ.

EXP.	CONSTITUTION	IND.	SPOTS	FREQUENCY IN % OF ALL SPOTS	% y SPOTS	y 1	y 2	y >2	sn^3 1	sn^3 2	sn^3 >2	+ 1	+ >2	+ ♂ COL.	$y\ sn^3$ 1	$y\ sn^3$ >2	y-sn^3 2	y-sn^3 >2	OTHER SPOTS
1a	$\frac{y + + Mn +^{bb}}{y\ w^e\ sn^3 + \theta}$	219	82	38	7	2	1	12	19	3	42	—	1	—	—	—	—	2	—
b	$\frac{y + + Mn\ bb}{y\ w^e\ sn^3 + \theta}$	1384	285	21	5	30	5	32	93*	26	91	—	—	—	—	—	1	5	2*
c	$\frac{y + + Mn\ bb'}{y\ w^e\ sn^3 + \theta}$	221+	59	27	10	8	1	14	15	8	10	1	—	—	—	1	—	1	—
	Totals Exp. 1				5.8	105			307			2		—	1		9		2
2a	$\frac{y\ w^e\ sn^3\ Mn +^{bb}}{y + + + \theta}$	97	6	6	5	3	—	2	—	—	—	—	—	1	—	—	—	—	—
b	$\frac{y\ w^e\ sn^3\ Mn\ bb}{y + + + \theta}$	260	13	5	3	2	1	6	1	—	—	1	2	—	—	—	—	—	—
c	$\frac{y\ w^e\ sn^3\ Mn\ bb'}{y + + + \theta}$	139	19	14	10	8	2	4	1	1	1	2	—	—	—	—	—	—	—
	Totals Exp. 2				5.6	28			4			5		1	—	—	—	—	—
3a	$\frac{y + + Mn\ \theta}{y\ w^e\ sn^3 + +^{bb}}$	857+	163	19	4	22	3	5	64	25	40	—	—	—	1	1	—	2	—
b	$\frac{y + + Mn\ \theta}{y\ w^e\ sn^3 + bb}$	95	16	17	2	2	—	—	3	3	8	—	—	—	—	—	—	—	—
	Totals Exp. 3				3.4	32			143			—	—	—	2		2		—
4a	$\frac{y\ w^e\ sn^3\ Mn\ \theta}{y + + + +^{bb}}$	557	41	7	5	16	7	4	5	2	4	1	—	2	—	—	—	—	—
b	$\frac{y\ w^e\ sn^3\ Mn\ \theta}{y + + + bb}$	348	29	8	4	12	—	3	2	3	3	—	1	5	—	—	—	—	—
c	$\frac{y\ w^e\ sn^3\ Mn\ \theta}{y + + + bb'}$	221	19	9	5	4	4	2	4	1	2	—	—	2	—	—	—	—	—
	Totals Exp. 4				4.6	52			56			2		9	—	—	—	—	—
	Totals Exp. 1–4	4398+	732																

* 1 spot showed thick heavy bristles of M length, the other was a sn^3 (very small seta) and sn^3 twin spot.

which occur in experiments involving Theta really results of crossing over between Theta and the X chromosome instead of results of crossing over between the X chromosomes and subsequent reductional separation of sister spindle points? (2) Do both the left and the right region of the Theta duplication cross over with their homologous regions in the X chromosome?

Four different combinations of *y*, sn^3, *Mn*, and Theta were investigated (table 16a). In all experiments eosin was involved also and in each of the four combinations different sub-groups were present with either the normal allele of bobbed, or two mutant alleles similar to each other, *bb* or *bb'*. (*bb'* has not as yet been described). No consistent differences between the sub-groups are apparent. The frequencies of total number of spots per hundred individuals varied between 5 and 38, but a consistent grouping is clear, giving the highest frequencies to experiment 1, the next to experiment 3, while experiments 2 and 4 present the lowest figures. Striking differences between the relative frequencies of the different types of spots, especially the sn^3 and *y* spots are also obvious. While in table 16a all *y* spots and all sn^3 spots were listed together regardless of their length, table 16b shows that among both groups there were spots with bristles of about normal length as well as of Minute length. An accurate determination of length in spots occurring on the abdomen or including only microchaetae was not possible in general, so that the majority of spots could only be classified in regard to *y* or sn^3. However, table 16b indicates that the great majority of spots were not Minute. Furthermore all 19 sn^3 Minute spots were single bristle spots so that it seems probable that all or most of the larger sn^3 spots were not Minute, and even among the single bristle spots the majority of the head-thorax spots were not Minute. This holds primarily for experiments 1 and 2. The bristles classified as not-Minute were

TABLE 16b

Spots (from table 16a) which could be classified according to seta length

EXP.	$y+^{M}$		$y\ M$		sn^3+^{M}		$sn^3\ M$		y-sn^3
	1	>1	1	>1	1	>1	1	>1	>
1	10	20	3	1	30	92	10	—	3*
2	—	6	-	1	1	2	--	—	—
3	1	1	-	--	8	22	8	—	2†
4	1	2	1	—		-	1	—	—

* $y+^{M}$-sn^3+^{M} 1 spot
$y+^{M}$–length of sn^3 not determinable 1 spot
$y\ M(?)$-length of sn^3 not determinable 1 spot
† sn^3+^{M}–length of *y* not determinable 2 spots

TABLE 17

Spots resulting from **x** *(equational) and* **y** *(reductional) segregation in experiments involving* y, sn^3, Mn *and* θ.

CROSSOVER REGION	EXP. 1 $\frac{y + Mn}{y\ sn^3 + \theta}$ (x)	(y)	EXP. 2 $\frac{y\ sn^3\ Mn}{y + + \theta}$ (x)	(y)	EXP. 3 $\frac{y + Mn\ \theta}{y\ sn^3 +}$ (x)	(y)	EXP. 4 $\frac{y\ sn^3\ Mn\ \theta}{y + +}$ (x)	(y)
(a) sn^3-Mn	$sn^3\ Mn$	+	$sn^3\ Mn$	+	$sn^3\ Mn$	y	$sn^3\ Mn$	y
(b) Mn-θ	sn^3	$y\ Mn$	+	$y\ Mn$	sn^3	$y\ Mn$	+	$y\ Mn$
(c) right end of θ and of X chromosome bearing θ	y(3X) dies		y(3X) dies		$y(M/M'+)$ $sn^3\ \underline{\theta\theta}$		$y(M/M'+)$ $sn^3/\underline{\theta\theta}$	
(d) right end of θ and of free X chromosome	$y(M/M+)$ $sn^3/\underline{\theta\theta}$	dies +(3X)	$y(M/M+)$ $+\ \underline{\theta\theta}$	dies +(3X)	y(3X) dies	$y(M/M'+)$ $sn^3\ \underline{\theta\theta}$	y(3X) dies	$y(M/M'+)$ $+/\underline{\theta\theta}$
(e) left end of θ and of X chromosome (3 possibilities)	$y\ Mn$		$y\ Mn$		$y\ Mn$		$y\ Mn$	
(f)	—		—		—		—	
(g)	$y\ Mn$		$y\ Mn$		$y\ Mn$		$y\ Mn$	

often found to be somewhat abnormal, slightly thickened or shortened in varying fashion. The causes for this will soon become clear.

If one tabulates for all four experiments the expectations for spots derived after different types of crossing over and segregation, a relatively simple explanation of the results appears. An abbreviated list of expectations is presented as table 17. Only single crossovers are considered and only the **x** and **y** type of segregation, the first being equational for the fibre points, the second reductional. The **z** type which is also equational does not lead to visible spots after single crossing over between the X chromosomes.

Process **a.** As the table indicates, crossing over between sn^3 and *Mn* with normal disjunction of the fibre points **ax** leads to sn^3 *Mn* spots in all four experiments. Non-disjunction of the fibre points **ay** leads to normal bristle spots in experiments 1 and 2 and to *y* not-*Mn* spots in experiments 3 and 4. No other types of crossing over and segregation represented in table 17 besides **ax** result in sn^3 *Mn* spots. These spots, in all four experiments, are therefore regarded as produced by the process **ax** (the actual non-occurrence in experiment 2 is probably due to the comparatively small number of flies inspected). In good agreement with the findings in the earlier experiments involving *y*, sn^3, and *Mn* is the fact that somatic crossing over to the left of *Mn* yields only single seta spots. This is interpreted as before to mean that crossing over away from the fibre region occurs only late in development.

The occurrence of the process **ay** can at most account for the + spots in experiments 1 and 2 and for the *y* spots in experiments 3 and 4. However it is more probable that these spots owe their existence to different processes to be discussed below.

Process **b.** Crossing over between *Mn* and the end of the X and equational segregation for the fibre points **(bx)** leads to sn^3 not-*Mn* spots in experiments 1 and 3, to normal bristle spots in experiments 2 and 4.

Spots which are sn^3+ may be expected also from some of the processes **c** and **d.** It is seen that segregation products in **c** and **d** will contain one X chromosome and two Theta duplications. Whole individuals of this constitution are not viable (unpublished results). In case hypodermal areas of this constitution are able to survive one would expect to find male sex indications in suitable spots. Actually, as will be shown soon, the great majority of sn^3+^M spots did not exhibit male characteristics. This leads us to the conclusion that most of the sn^3+^M spots in experiments 1 and 3 owe their origin to the process **bx.** One would expect a high corresponding number of normal bristle spots in experiments 2 and 4. Only few such spots were found although their relative frequencies in experiments 2 and 4 are higher than in experiments 1 and 3. However, on account of the great variability in the occurrence of larger sized spots in the head-thorax region in different experiments no serious obstacle is seen in this lack of normal spots which are only visible under the conditions just stated. And it is significant in this respect that of the 100 *y* or sn^3 spots in experiments 2 and 4 only 15 could be classified in regard to seta length. While this type of crossing over to the right of *Mn* with normal segregation can account for the majority of sn^3 not-*Mn* spots, non disjunctional segregation of the fibre points **by** can at most account only for the small number of *y Mn* spots and other processes will also yield this type of spot.

Processes **c, d.** An explanation for the great number of $y+^M$ spots and their varying percentage in the different experiments is provided by the assumption of crossing over between the right end of an X chromosome and the homologous part of the Theta duplication. Two main types of crossovers are possible and assumed to occur with equal frequencies: process **c,** crossing over between Theta and an X chromatid with the Theta duplication; and process **d,** crossing over between Theta and a free X chromatid.

It is seen that normal disjunction **(x)** of the fibre points after crossing over **c** in experiments 1 and 2 yields triple-X *y* spots which possess *Mn* in one of the three X chromosomes. Such a constitution *M*/+/+ is known to produce not-Minute bristles, while the twin segregate whose single X chromosome contains *Mn* is inviable. In experiments 3 and 4 crossing over **c** and normal segregation leads to sister cells, one with 3 X chromosomes and one with one X chromosome and two Theta duplications. The cell with three X chromosomes carries two *Mn* factors. As individuals of such constitution as well as individuals with one X and two duplications are not viable it is assumed that hypodermal segregation products of these constitutions will at best be of low viability and only rarely give rise to spots. No reductional segregation for the spindle fibre points is expected to occur after crossing over of type **c,** as this would mean non-disjunction of the two complete non-crossover sister chromatids which do not carry Theta, a process which throughout this paper has been shown either not to occur at all or at best as an extremely rare exception.

Crossing over **d** with equational segregation **(x)** in experiments 1 and 2 leads to the similar types of inviable or barely viable twin cells with triplo-X *Mn*/*Mn*/+ and $X+^M$ cells with two Theta duplications, but in experiments 3 and 4 to triplo-X yellow *Mn*/+/+ cells and inviable X *Mn* partners.

Reductional segregation **(y)** leads to triplo-X cells with normal appearing bristles in experiments 1 and 2 and to combinations with low viability in experiments 3 and 4. Spots with these phenotypes are of course to be expected also from other processes.

Altogether equational segregation after crossing over **c** leads to *y* spots in experiments 1 and 2 and after crossing over **d** to *y* spots in experiments 3 and 4. The triplo-X *Mn*/+/+ constitution of these spots agrees with the somewhat abnormal appearance of the *y*-bristles, the triplo-X condition making for heavier than normal bristles, the one *Mn* possibly accounting for the slight decrease in bristle length.

The frequencies of *y* spots in experiments 1 and 2 should be higher than in experiments 3 and 4 depending on the degree of viability of triplo-X *Mn*/*Mn*/+ cells. If this viability is zero, the percentage of *y* spots in experiments 1 and 2 would be twice that in experiments 3 and 4, for one of the two normal disjunctional types of segregation, **(z)**, does not lead to visible spots in experiments 3 and 4 while it is identical in effect with **x**-segregation in experiments 1 and 2. The actual average frequencies are 5.7 per cent for experiments 1 and 2 and 4.0 per cent for experiments 2 and 3. (These figures are based on total *y* spots and are not corrected for the presence of *y Mn* spots.) Calculation shows that such a difference would follow if the chance of finding a *y Mn*/*y Mn*/*y* spot is about one-third of that of finding a *y Mn*/*y*/*y* spot. While this result points to a definite viability of triplo-X spots with two *Mn* factors, not too much weight should be attached to the value obtained.

Processes **e–g.** Finally we have to consider the possibility of crossing over between the left end of Theta and the homologous left end of the X chromosomes. Assuming only equational fibre segregation, two of these processes, **e** and **g,** can lead to the production of *y Mn* spots, while process **f** will not yield visible spots. This represents an alternative to the reductional process which also can account for *y Mn* spots. As the occurrence of reductional somatic segregation has been excluded or made improbable in other cases, it is assumed that the *y Mn* spots are products of equational segregation following the processes **e** and **g.**

Summarizing, we can state that equational segregation of fibre points after single crossing over between the X chromosomes in the region from *Mn* to fibre point and crossing over between the right end of an X chromatid and Theta with equational segregation can account for the great majority of spots, namely the sn^3+^M and $y+^M$ spots. Crossing over with equa tional segregation occurs also to the left of *Mn*, at a low frequency, as witnessed by the sn^3Mn spots which are products of crossing over in the sn^3 to *Mn* region; proof of crossing over in the w^e to sn^3 region is seen in the occurrence of one out of four sn^3+^M spots which all enclosed eye or ocellar areas but of which the one did not show eosin eye color.

Crossing over between the homologous left regions of the X chromosome and the Theta duplication with equational fibre segregation is assumed to occur and to account for the *y Mn* spots. No detailed attempt will be made to explain the origin of the remaining classes of spots, which all have few representatives only. Multiple crossing over and occasional new crossover processes in descendants of crossover cells will account for them as it will also for a fraction of the main classes.

The high frequency of crossing over between Theta and the homologous right end of the X chromosomes is surprising in view of the rareness of this process in germinal crossing over. In order to get an estimate, however inaccurate, of this frequency the data of experiment 1 may be considered. The frequencies of *y* spots and of sn^3 spots are 105 and 307. A certain percentage of the *y* and of the sn^3 spots is *Mn*. If the figures of table 16b are taken as a basis for estimating the $y+^{M}$ and $sn+^{M}$ spots—a procedure which is doubtful if viewed in the light of our findings concerning the different ontogenetic time at which different crossover processes occur, we find $(30\div 34)105=93$ $y+$ spots and $(122\div 132)307=284$ sn^3+^{M} spots or about 1 $y+$: 3 sn^3+. This means that out of each four crossovers in the right end regions one involves the Theta duplication. This is considerably higher than the relative frequency of Theta crossovers in similar experiments without the presence of *Mn* (table 13). However it is still far from what would result from chance crossing over within a multivalent between the four right ends of the X chromosomes and the two Theta duplications. Excluding sister strand crossing over there would be the following types of crossing over: 4 cases of "X with X"; 4 cases of "free X with Theta"; 2 of "duplication-X with the one Theta duplication"; 2 of "duplication-X with the sister Theta duplication." Assuming normal disjunction of fibre points in 50 per cent of the first group, sn^3+^{M} spots would result. No visible spots would occur in consequence of crossovers according to the second and third group, but in all cases of the last group $y+^{M}$ spots would be produced. Chance pairing thus would lead to a 1 : 1 proportion of y : sn^3 spots instead of the observed 1 : 3 relation.

It is probably significant that crossing over involving Theta is rare in germinal cells where there is a high frequency of crossovers away from the attachment region, while such crossing over occurs frequently in somatic crossovers of flies without *Mn* (table 13) where crossing over becomes concentrated in the proximal region. It is significant that Theta crossing over is relatively still more frequent in the present experiments where, under the influence of *Mn*, the shifting of crossing over to the attachment region has been increased.

As judged by the rareness of *y Mn* spots the frequency of crossing over involving the left end of the Theta duplication is considerably lower than that involving the right end of the duplication.

In table 18 the information as to the sex of spots in this series of experiments is presented. At least 21 out of 25 sn^3 spots were female, as was to be expected after crossing over between the X chromosomes. All three critical y spots were probably female in constitution again as expected, as was one w^e-colored eye-spot which did not affect any setae. Besides these female spots, however, 4 sn^3 male spots and 10 male-colored spots with normal setae were found. It seems probable that these spots represent occasional survivals and division products of 1X cells with two Theta duplications produced from crossing over between the right end of an X chromosome and Theta. The same explanation applies to at least two of the three male-colored spots recorded in line 4 of table 15.

TABLE 18

The sexual coloration in critical spots from experiments involving y, sn^3 Mn, and θ (see tables 13, 17)

EXP.	sn^3 (ABDOMINAL)		w^e sn^3		+	w^e	y	
	NO ♂ COL	♂ COL	NO ♂ COL.	♂ COL.	♂ COL.	♀ COL.	NO ♂ COL	♂ COL
1a	3	—		1		—	—	—
b	15	2		1	—	—	—	—
c	1	—	—	—	—	—	—	—
2a		—	—	—	1	—	—	—
3a	2		—	—	—	1*	—	—
4a	—	—	—	—	2		—	—
b	—	—	—	—	5	—	1(?)	—
c	—	—	—	—	2	—	2(1?)	—
Total	**21 ♀**	**2 ♂**	—	**2**	**10**	**1**	**3**	—

* No setae affected.

It is in agreement with this assumption that the sn^3 male spots are not found in experiments 2 and 4 where they are not expected and that likewise the + male spots are not found in experiments 1 and 3 where they should not occur. The very small numbers of critical spots in experiments 2 and 3 diminish somewhat the weight of these facts.

The analysis of this group of experiments furnishes independent evidence of:

(1) the occurrence of equational segregation of the fibre points as the main, if not the only type;

(2) the frequent occurrence of somatic crossing over between the homologous right regions of the Theta duplication and the X chromosomes;

(3) the lower frequency of somatic crossing over between the homologous left regions of the Theta duplication and the X chromosomes and

(4) the low frequency of crossing over away from the fibre point region in experiments involving *Mn*.

Points (2) and (4) are probably connected causally.

AUTOSOMAL MOSAICS

Somatic autosomal crossing over and segregation

As has been referred to earlier, spots affecting autosomal characters have been found to occur in flies which carry autosomal Minutes. In a preliminary communication (STERN 1927b) an explanation for these spots was offered which was modeled after the hypothesis of chromosome elimination. A reinterpretation is now necessary in the light of our present knowledge regarding somatic segregation. The experiments to be discussed concern mosaic spots which have occurred in flies heterozygous for the third chromosome dominant Minutes *My*, *Mw*, and *Mβ*. The spots were recognized as areas exhibiting the phenotype of recessive alleles which were present in the zygote in the heterozygous state only. No third chromosome

TABLE 19

Frequencies of autosomal spots in experiments with My, Mw, Mβ, and M33j

CONSTITUTION	*M*-INDIVIDUALS ♀	*M*-INDIVIDUALS	*M*-INDIVIDUALS ♂	SPOTS ♀	SPOTS ♂	SPOTS SEX?	SPOTS %	+ INDIVIDUALS ♀	+ INDIVIDUALS	+ INDIVIDUALS ♂	SPOTS
(1) *My/ru h th st* p^p *sr* e^s	2757		2564	42	24	—	1.2	3837		3495	—
(2) *My/Sb*		980		8	2	2	1 2	—		—	—
(3) *Sb Mw/ru h th st cu sr* e^s *ca*	1348		1526	46	38	- -	2 8		-		
(4) *Mw/Sb*		419				3	0 7		461		3*
(5) *Mw/ru h th st* p^p *cu sr* e^s	580		647	3	7	1	0 8	-		--	-
(6) *Mw/h st th sr* e^s *ro ca*	583		525	4	1		0 4				-
(7) *Mβ/ru h th st sr* e^s *ca*	1487		1240	6	1		0 3	1579		1345	
(8) *M33j*/+(6 separate experiments)	1915		—	1	--	-	0 05	--		-	-
Total ♀ (excl. exp. (2) (4) (8))	**6755**		—	**110**	-	+(?)	**1.6**	**5416**			- -
Total ♂ (excl exp. (2) (4) (8))	-		**6502**	-	**73**	+(?)	**1.1**	- -		**4840**	—
Grand total	—	**16571**	--	—	**189**	—	**1.1**	**10717**		—	**3**

* Spot on ♀ : 1.
Spot on ♂ : 1.
Spot on sex unknown: 1
All these had $+^{Sb}$ setae. In one of them the setae were very small in addition.

mutants which are distinguishable in every single micro- or macrochaeta, like the sex-linked mutants *y* and sn^3, were available but the absence of the dominant Minute is recognizable in a single macrochaeta as is the absence of the dominant gene Stubble (*Sb*, III, 58.2). The recognition of the other genes used depends on the occurrence of multicellular areas in certain regions of the head and thorax.

In comparing the frequencies of autosomal spots (table 19) with those of sex-linked mosaic areas it is necessary to remember the different probabilities of discovering spots which become visible whenever they include a seta and spots which are phenotypically apparent under certain conditions only. It is, however obvious that the frequency of third chromosome spots is considerably lower than that of comparable sex-linked spots in flies containing *Mn* or the Blond-Minute.

A similar difficulty arises in an evaluation of the frequency of autosomal spots in Minute flies as compared to that of controls which contain no Minute factor. As can be seen from tables 20 24, a large number of spots in Minute flies were recognizable only by the appearance of $+^M$ bristles In not-Minute controls such areas would not be different from the rest of the fly. It is certain that on the whole the presence of autosomal Minutes increases the incidence of autosomal spots as shown by the fact that no spots were found in 7332 not-Minute individuals, controls of experiment 1, nor in 2924 control flies in experiment 4. However, among the *Sb*/+ controls of experiment 4 three individuals occurred each of which possessed one not-Stubble bristle.

Of the three Minute factors studied, the locus of *My* is to the left of the fibre attachment point at 40.4, while that of *My* and *Mβ* is to the right of this point, at 80± and 85.4 respectively.

My. Two experiments involved *My*. In the first, *My* was present in one chromosome, while the following recessives were present in the homologous third chromosome: *ru*, 0.0 roughoid; *h*, 26.5 hairy; *th*, 42.2 thread; *st*, 44.0 scarlet; p^p, 48.0 peach; *cu*, 50.0 curled; *sr*, 62.0, stripe; e^s, 70.7, sooty. Phenotypically, such flies show only the Minute bristle condition. Table 20 indicates that 66 spots were found. In all cases (57 out of 66) where the spots enclosed one or more macrochaetae it was seen that these setae were normal in length, indicating the absence of *My*. Whenever the spots covered an area which in a fly homozygous for *h* would exhibit the extra-hair effect of this recessive gene extra hairs were present indicating the absence of the normal allele of *h* (45 cases). In 8 out of 9 cases, where the spot involved part of an eye, the recessive characters roughoid eye-texture and scarlet eye-color were visible, indicating the absence of the normal alleles of these genes. The arista of one antenna was involved 6 times and presented the phenotype of "thread," indicating the absence of this gene's normal allele. In contrast to the appearance of the phenotypes of *ru*, *h*, *th*, *st*, and $+^{My}$ no spot presented the phenotypes peach eye-color and stripe and sooty body coloration. Peach would have been recognized in the 9 spots which covered part of an eye and sooty or stripe and sooty would have been recognizable in more than 20 spots. The phenotype curled wing did not occur either, but it is unknown if this is an autonomous character which would appear in mosaic spots.

While the foregoing description has taken account of each gene by itself, many spots in reality permitted the determination of the presence or absence of more than one character at the same time. This is to a certain degree obvious from an inspection of table 20. As a whole the findings show that spots produced in *My* flies exhibit all those recessive genes which are located in the $+^M$ carrying third chromosome to the left of a point between *st* and *p* and that the spots do not exhibit phenotypes of any genes located to the right of this point. The only exception is represented by one spot which covered part of the head and showed the phenotype *h*, *st*, and $+^M$ but not that of *ru*.

TABLE 20

Spots on My/ru h th st p^p sr e^s flies (cf. table 17).

$h+^M$		$ru\ h\ th\ st+^M$	$ru\ h\ st+^M$	$ru\ st$	$h\ st+^M$	$+^M$		h	th
1	>1	>2	>2	0	>2	1	>1	0	0
2	32	3	2	3	1	12	5	5	1

The second experiment which involved *My* concerned flies of the constitution *My/Sb*, with the phenotype Minute-y Stubble bristles (table 21).

TABLE 21

Spots on My/Sb flies (cf. table 17).

$+^M Sb$		$M+^{Sb}$		(?)$M\ Sb$
1	>1	1	>1	1
3	6	2	1	1

There were 13 aberrant areas. In 9 cases the phenotype *My* had disappeared, while that of Stubble was still present, in 3 cases the reverse was true, resulting in Minute, not-Stubble bristles. The 13th spot was formed by one bristle, which showed both Minute and Stubble characters although in abnormal fashion.

Mw. Before we discuss these mosaic spots induced by Minute-y, we shall describe the phenomena in flies possessing Minutes located in the right arm of the third chromosome. The largest experiment involved Minute-w, *Sb*, and the recessives *ru*, *h*, *th*, *st*, *cu*, *sr*, *e^s*, *ca* (*ca*, III, 100.7

claret; table 22: *Sb Mw/ru h th st cu sr* e^s *ca*). In 73 out of the total of 84 spots both *Sb* amd *Mw* had disappeared. Of these 73 spots 14 were large enough and were situated in suitable regions so that the presence or absence of e^s or e^s and *sr* was recognizable; indeed, these genes, originally present in heterozygous condition only, were phenotypically obvious in all 14 cases, as well as in 4 more areas (not included in the 73 areas) which did not involve macrochaetae, so that their nature in respect to *Sb* and *Mw* could not be tested. Two $+^{Sb}+^{M}$ spots involved part of the eye and were claret-colored. No single spot exhibited the phenotypes *ru*, *h*, *th*, or *st*, although *ru* and *st* would have been recognizable in 2 spots, *h* in at least 9 spots, and *th* in 1 spot. In total, 73+4=77 spots can be interpreted as exhibiting when possible the phenotypes of all recessive genes located in the $+^{M}$ carrying third chromosome to the right of a point between *st* and *Sb*, and they do not exhibit phenotypes of any genes located to the left of this spot. These findings are completely opposite to the findings regarding *My*.

TABLE 22

Spots on Sb Mw/ru h th st cu sr e^s *ca flies (cf table 17).*

$+^{Sb}+^{M}e^{s}$(or e^{s} *sr*)	e^{s} or e^{s} *sr*	$+^{Sb}+^{M}ca$	$+^{Sb}+^{M}$		$Sb+^{M}ca$	$Sb+^{M}$		$+^{Sb}M$
	0	>1	1	>1	>1	1	>1	1
14	4	2	2	55	1	2	2	2

There occurred 7 exceptional spots. They agreed with the rest by not exhibiting any genes located to the left of the region noted (1 case: $+^{ru}+^{h}$), but differed by showing either $+^{Sb}M$ or $Sb+^{M}$ bristles and in addition neither *sr* nor e^s (1 case).

A corroboration of these results is provided by 11 spots in flies of the constitution *Mw/ru h th st* p^p *cu sr* e^s and 5 spots in flies of the constitution *Mw/h th st sr* e^s *ru ca* (table 23). In 13 cases the recessive genes of the right half of the $+^{M}$ third chromosome were visible ($+^{M}$, e^s, or *sr*, e^s according to the region involved). In 6 out of these 13 cases the phenotypic absence of the left arm gene *h* was evident although its presence would have been recognizable. While these results agree with the general deduction which has just been made, the remaining 3 mosaic areas were exceptional again. They did not exhibit the phenotypes *sr* and e^s, in spite of location on suitable thorax regions. In two of these spots it could be ascertained that *h* did not appear. This finding agrees with that in all other spots.

A last experiment involving *Mw* concerned flies of the constitution *Sb/Mw* (419 individuals). Only 3 spots were found, two of which were phenotypically $Sb+^{M}$ (1 doubtful), the third being $+^{Sb}M$.

An experiment in which *M β* was present is reported in table 24. Of a

TABLE 23

Spots on Mw flies (cf table 17)

CONSTITUTION	$+^{M}$ e^{s} (or e^{s} *sr*)		e^{s} or e^{s} *sr*	$+^{M}$		$+^{M}+^{e\ sr}$
	0	>2	0	1	>1	>1
Mw/ru h th st p^p cu sr e^s	3		4	1	1	2
Mw/h th st sr e^s ro ca	1	2	—	1		1

total of 7 spots 6 exhibited suitable recessive genes located in the right arm of the not-Minute third chromosome, while no spot with genes located in the left arm appeared (1 spot was definitely $+^{h}$). The seventh mosaic area was exceptional in having $+^{M}$ bristles but also $+^{sr}$, $+^{e}$ coloration (*h* did not appear either).

All findings regarding autosomal mosaic areas in flies carrying third chromosome Minutes can be summarized in the following statements:

(1) Spots exhibit only phenotypes of recessive genes, which are located in one arm of the not-Minute carrying chromosome. If the Minute factor is in the left arm of the third chromosome (*My*), only recessive genes in the left arm become visible; if the Minute factor is located in the right arm (*Mw*, Mβ), only recessive genes in the right arm exhibit their effect.

(2) In the majority of spots all genes located in the respective arm are involved.

(3) A smaller number of spots exhibits only the phenotypes of *some* genes located in one arm.

✓Originally these findings were explained by the elimination assumption. That arm of the Minute-bearing chromosome or part of it in which the Minute is located was thought to be broken off and lost from the cell. The following considerations make this assumption untenable now: (a) the one spot reported in table 20, which exhibited *h*, *st*, and $+^{M}$, but not *ru* can be explained by elimination only if one assumes the loss not of a whole arm or a whole end section of the chromosome but of a middle piece. Such a process would necessitate special assumptions with regard to elimination which seem rather artificial. It should be mentioned, however, that a slight possibility exists that the absence of the phenotype *ru* in the eye-spot may not really indicate the presence of the dominant $+^{ru}$ but may conceivably be an extreme phenotypic variation of the somewhat variable expression

TABLE 24

Spots in Mβ/ru h th st cu e^s ca flies (cf. table 19).

$+^{M}$ e^{s} (or e^{s} *sr*)	e^{s} or e^{s} *sr*	$+^{M}$	$+^{M}+^{e,sr}$ ($+^{h}$)
2	3	1	1

of *ru*. (b) As will be shown below, the occurrence of autosomal spots can be interpreted by the theory of somatic crossing over in autosomes. As the theory of somatic crossing over has been proven to be superior in the case of sex-linked mosaics, it is a priori probable that it holds also for autosomes. A direct test which would be provided by a demonstration of autosomal twin spots cannot be given, since autosomal spots occur hardly at all in not-Minute flies, while in Minute flies one of the segregation products after somatic crossing over becomes homozygous for the Minute factor and is not viable. Thus only a single, not a twin, mosaic area can appear.

Granting the validity of the theory of autosomal somatic crossing over and segregation, a detailed analysis of the data yields an unexpected result. We consider first those cases which form the majority and which demonstrate that the region of somatic crossing over is restricted to the neighborhood of the spindle fibre point. This point in the third chromosome is located in the middle, between scarlet (44.0) and pink (48.0, peach is an allele of pink). It was just this interval in experiment 1 (table 20) which divided the genes whose phenotypes appear in mosaic areas from those which did not appear; in experiment 3 (table 22) where the critical region could not be narrowed down as completely, but could only be said to lie within the interval *st* (44.0) to *Sb* (58.2) the spindle fibre point is again included. Restricting our analysis to single crossovers between two of four strands only, there are four possible crossing over and segregation processes which lead to the appearance of somatic areas:

(1) *Crossing over to the left of the fibre point.*

(x) If *A B* S' *C D a b* S^2 *c d* is the original constitution of the autosomes, S' and S^2 representing homologous fibre points, segregation of the four chromatids after crossing over with normal equational separation of the daughter fibres **(x)** yields: *A B* S' *C D/A B* S^2 *c d* and *a b* S' *C D/a b* S^2 *c d*. The latter constitution would be visible as a mosaic area exhibiting *a* and *b*.

(y) Non-disjunctional separation of the daughter fibres **(y)** yields *A B* S' *C D/a b* S' *C D* and *A B* S^2 *c d/a b* S^2 *c d*, the latter exhibiting *c* and *d*.

(2) *Crossing over to the right of the fibre point.*

(x) segregation results in *A B* S' *C D/a b* S^2 *C D* and *A B* S' *c d/a b* S^2 *c d*, yielding a spot *c d*.

(y) segregation results in *A B* S' *C D/A B* S' *c d* and *a b* S^2 *C D/a b* S^2 *c d*, yielding a spot *a b*.

An *a b* spot therefore occurs either as a consequence of crossing over to the left of the fibre point and equational fibre segregation (process **1x**) or as a consequence of crossing over to the right and non-disjunctional separation (process **2y**). Correspondingly a spot *c d* occurs either in consequence of crossing over to the right of the fibre point and equational fibre segregation (process **2x**) or after crossing over to the left of the fibre point and non-disjunctional separation (process **1y**). It is believed that only one of each alternative is actually realized and that this involves normal equational separation of daughter fibre points. Reasons for this assumption are (1) the analogy with somatic segregation of X chromosomes in which equational separation has been demonstrated to be the rule and (2) the demonstration to be given in the next paragraph that equational separation of fibre points is the only method followed after somatic crossing over distal to the fibre region in the third chromosome.

An analysis of the smaller group of spots which exhibit only the phenotypes of some genes located in one third chromosome arm leads to an interpretation which considers these areas as caused by consequence of crossing over in regions other than that including the spindle fibre point. Single crossovers are sufficient to yield the different spots reported in columns 6, 7 and 8 of table 22, and the last columns of tables 23 and 25. All these spots occurred in experiments which involved Minute factors in the right arm of the chromosome. The locus of crossing over can be placed to the right of *e* (70.7) and to the left of *Mw* (80.0) or *Mβ* (85.4) respectively; this is judged from the fact that all recessive loci to the left of the *e-M* region failed to appear in mosaic areas, while those to the right did appear. Non-disjunctional segregation would have yielded areas which exhibit all recessive genes located to the left of the crossover point, thus including loci from both arms of the chromosome. But no such areas did occur. It can be demonstrated that these cases of somatic crossing over can have been followed only by equational fibre point segregation which would lead to the observed types of spots.

There remains a discussion of the few spots not yet dealt with. If the one $h\ st+^{M}$ spot of table 20 can be regarded as genotypically $+^{ru}$ as indicated by its phenotype, a case of double crossing over would be represented, the crossover points being located in the regions *ru-h* and *st-p*. A double crossover between the fibre point and *Sb* and between *Sb* and *Mw* would explain the $+^{Sb}Mw$ spots of table 22 and the $+^{Sb}Mw$ spot mentioned in the last paragraph of the section on *Mw*. The $My+^{Sb}$ areas recorded in table 21 can be regarded as crossover products to the right of the fibre point. Such spontaneous crossovers are believed also to be the explanation of the occasional occurrence of $+^{Sb}$ spots in not-Minute flies (table 19 (4), control). An alternative explanation assuming crossing over to the left of the fibre point would demand non-disjunctional fibre segregation. For the exceptional spot recorded in the last column of table 21 and the $+^{Sb}$ *M*-like spot among the controls of experiment 4, table

19, no discussion of hypothetical interpretations seems warranted. The unexpected result brought out in the analysis of areas involving third chromosome genes consists in the definite correlation between the location of the Minute factor in the left or right arm of the chromosome and the corresponding location of the crossover point. No well-founded theory of this relation can be given.

In addition, it seems noteworthy that the distribution of crossovers along the chromosome is greatly changed as compared to germinal crossing over. The great majority of somatic crossovers seems to fall within the interval *st-p* which comprises less than four per cent of all germinal crossovers. As this interval encloses the fibre attachment point it represents the region adjacent to this point. The concentration of somatic crossovers in these regions parallels our former findings concerning the massing of somatic crossovers in the region adjacent to the fibre point of the X chromosome.

The occurrence of autosomal crossing over in females and males ✓

Autosomal mosaic areas appeared both on females and on males. Somatic crossing over in autosomes is thus shown to occur in both sexes. However, it is seen from table 19 that a probably significant lower frequency was found in the males.

It may be added that an experiment was made to test the question whether an autosomal Minute would induce crossing over in its chromosome in the germ cells of males. Males of the constitution *Mw/h st cu, sr,* e^s *ca* were backcrossed to homozygous *h st cu sr* e^s *ca* females. None of the 4151 F_1 individuals were crossovers (class *Mw* = 2092, class *h st cu sr* e^s *ca* = 2059). There is then either no effect of *Mw* or the effect is too small to become apparent in this sample of more than 4000 flies. The experiments on induction of crossing over in the male of *Drosophila melanogaster* by Friesen (1934), Patterson and Suche (1934) and A. F. Shull and Whittinghill (1934) by means of X-rays or heat yielded positive results with much smaller numbers.

MOSAIC AREAS IN FLIES HETEROZYGOUS FOR X CHROMOSOME INVERSIONS

Whenever an X chromosome was present in our experiments which carried the bobbed-deficiency, described by Sivertzev-Dobzhansky and Dobzhansky (1933), unexpected peculiarities relating to mosaic areas were found. Attempts at interpretation met difficulties until it became known that the bb^{Df} chromosome, besides lacking a section of a normal X chromosome, carries a long inversion with the end points at 9± and

TABLE 25a

Spots in experiments involving primarily y, sn^3, and bb^{Df}. No Y chromosome present.

EXP.	CONSTITUTION*	IND.	SPOTS	NO SPOTS	sn^3			y			$y\ sn^3$			y-sn^3		y-$sn^3\ y$	+♂ COL.
					1	2	>2	1	2	>2	1	2	>2	2	>2	2	
1a	$y\ bb^{Df}/sn^3$	884	12†		6	—	2	4	—	—							
		340	28		8	2	—	12	3	3							
		683	153		34	8	11	62	16	10				4	7		1
		122	18		2	—	1	7	3	2				2	—		1
					50	10	14	85	22	15				6	7		
Totals Exp. 1		**2029**	**211**	**10**	**74**			**122**			—			**13**			**2**
2	$y\ sn^3\ bb^{Df}/+$				2	—	—	6	3	3	19	1	1			1	1
		838	37	4	2			12			21			—		1	1
3a	$sn^3\ bb^{Df}/y$				23‡	1(?)	—	14	4	2	—			—		—	—
		821	44		24			20									
		22	20		3	1	—	8	4	—	—			3	1	—	—
					4			12						4			
		29	6		2	—	1	—	1	1	—			—	1	—	—
					3			2						1			
				8§	28	2	1	22	9	3				3	2		
Totals Exp. 3		**872**	**70**	**(6)§**	**31**			**34**						**5**			
4	$bb^{Df}/y\ sn^3$	740	15	2	1	—	(1?)	3	2	1	1	1	4	—	—	—	1
					2			6			6						

* Besides the genes indicated, other recessive sex-linked factors were present in heterozygous condition in some experiments. An autosomal Minute was present in some of the flies of exps. 1a, b, c. In exp. 3c and partly in exp. 4 the constitution in respect to the *bb* locus was bb^{Df}/bb.

† Inspected for head and thorax spots only.

‡ Two doubtful.

§ The value of 6% is obtained if one excludes exp. 3b which represents individuals of one single culture which, raised at the same time with 29 cultures recorded as exp. 3a, gave an exceptionally high number of *y* spots.

64± (BEADLE and STURTEVANT 1935). On the basis of this fact an explanation of the observed spots could be given. Furthermore, in the course of working with flies containing bb^{Df} it was found that bb^{Df} stocks frequently contained supernumerary Y chromosomes as had been described for an independent bb^{Df} stock by GERSHENSON (1933). This led to the discovery of a striking influence of the Y chromosome on somatic crossing over. Accordingly the results for each experiment involving a known constitution of the X chromosomes will be given separately for those cases in which no Y chromosome was present in the females and those in which a Y chromsome was present. Special crosses had to be undertaken in order to assure the absence or the presence of an extra Y chromosome and in many cases progeny tests were carried out as independent tests.

Experiments involving y, sn^3, and bb^{Df}; no Y chromosome present

As in foregoing analyses, all four possible combinations of the three allelic pairs were secured so that any interpretation has to stand a fourfold test. The results are given in tables 25a and b. If we compare them with the similar experiments involving *y* and sn^3 recorded in tables 5 and 8 we note (a) the relatively low number of spots with 2 or more setae, (b) the relatively low number of twin spots in experiments 1 and 3, (c) the relatively high number of spots other than *y* sn^3 in experiments 2 and 4. A further very striking fact is not indicated in table 25: although the setae in non-mosaic areas were of normal size, the majority of *y* sn^3 setae in experiment 2 and of the sn^3 setae in experiment 3 were of very small size varying from fine, thin structures to larger but still distinctly subnormal ones. A similar picture was presented by many *y* setae in experiment 1. It was different with at least part of the *y* setae in experiments 2 and 3, as well as with most of the different setae in experiment 4; they were of normal size.

The explanation of these phenomena is based on MCCLINTOCK'S (1933) cytological findings with reference to crossing over involving chromosome inversions. Her results have been confirmed by later workers (MÜNTZING 1934; SMITH 1935; MATHER 1935; HÅKANSSON 1936) and have been applied to a genetic analysis by BEADLE and STURTEVANT (1935). We shall restrict our discussion to the consideration of single crossovers and equational fibre point segregation, processes which can account for the great majority of spots. Crossing over can occur in two different sections of the X chromosome, outside the inverted region and inside. The first section in a chromosome carrying the bb^{Df} inversion is formed by the left end of the X chromosome, from *y* to about the locus 9, the second section by the rest of the chromosome. Whenever somatic crossing over occurs between the left ends of the chromosomes we obtain the same results as discussed

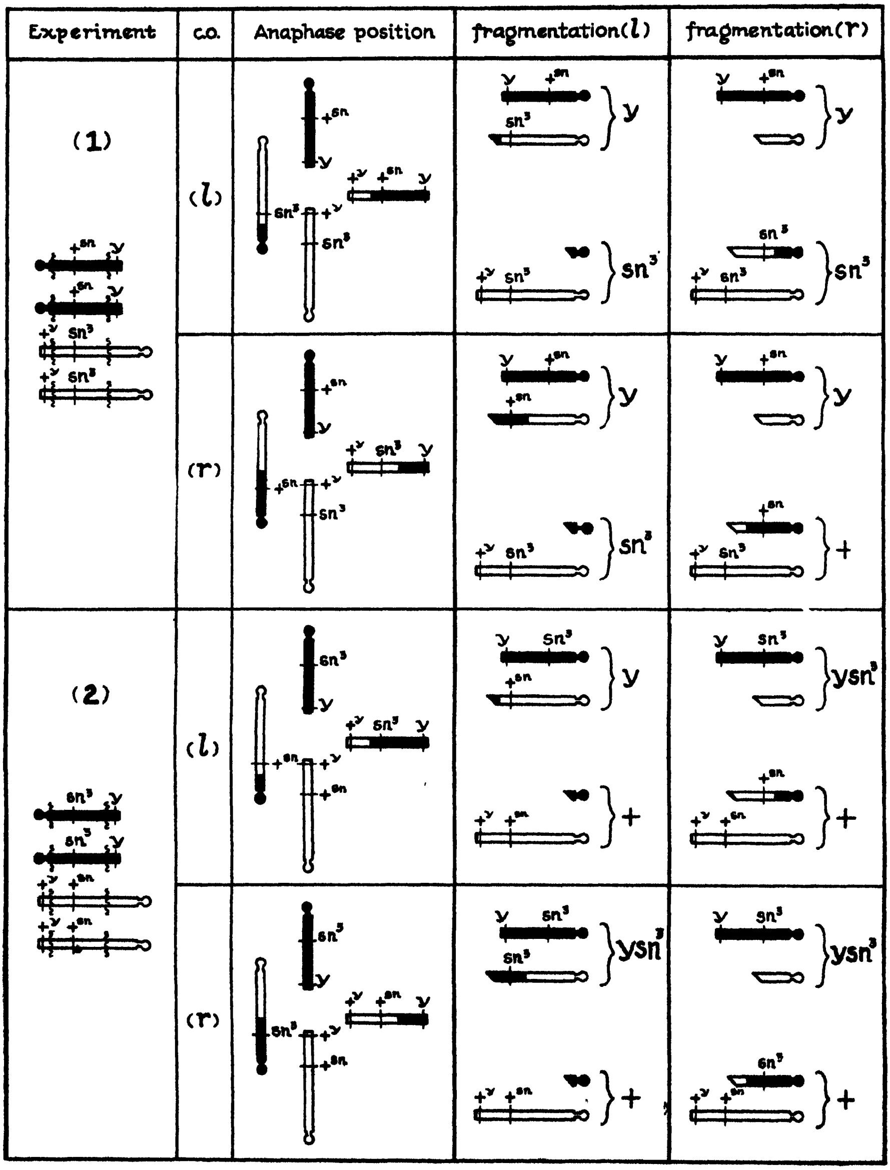

FIGURE 10 a, b. Four experiments involving y, sn^3 and bb^{Df}. (See tables 25–27, experiments 1, 2, 3, 4.) The results of x-segregation after crossing over to the left (l) and to the right (r) of sn^3 and of fragmentation of the chromatid with two fibre points to the left (l) and to the right (r) of sn^3. The lines ⌇ indicate the end points of the inverted region.

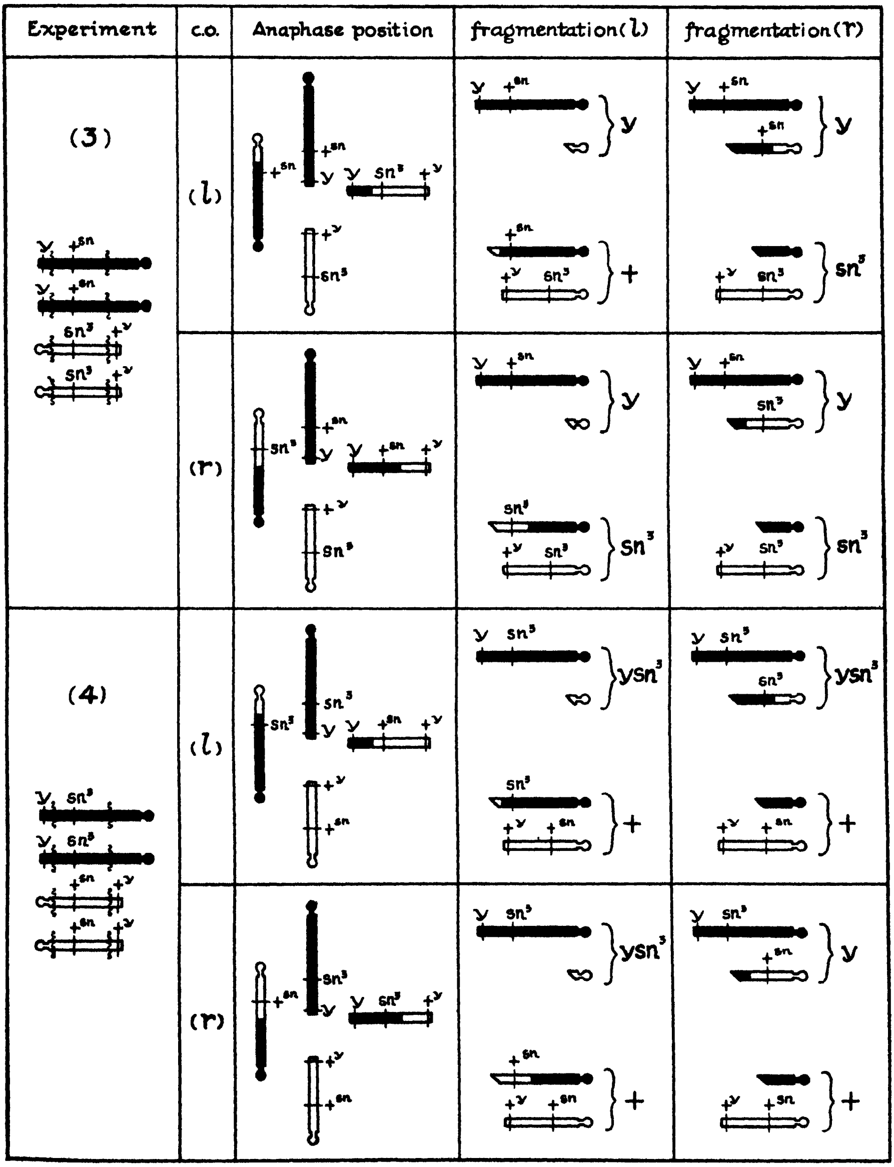

FIGURE 10b (see opposite)

in an earlier chapter, namely, in all four experiments, the appearance of single *y* spots. The spots contain two X chromosomes and the phenotype of these *y* setae is expected to be normal. Part of the *y* spots with setae of normal length are believed to have originated in this way.

A very different situation is produced as a consequence of somatic crossing over within the inverted region (fig. 10). If crossing over occurs at a four strand stage, two normal non-crossover strands will be formed, while the two other strands will yield one strand containing no spindle fibre point and one containing one fibre point on each end (fig. 10). If segregation is accomplished by equational disjunction of sister fibre points, two possible distributions result.

TABLE 25b

Sexual coloration of critical spots from table 25a.

EXP.	*sn*³		*y*		*y sn*³	+	TOTAL	
	NO ♂ COL.	♂ COL.	NO ♂ COL.	♂ COL.	♂ COL.	♂ COL.	NO ♂ COL.	♂ COL.
1b	1	—	—	—	—	—	15	3
c	5	2	9	—	—	1		
2	—	—	1	—	—	1	1	1
4	—	—	1	—	1	—	1	1
Totals							17	5

(1) Segregation **(z)**. Both non-crossover strands go to one pole and the crossover products to the other pole. The chromatid without any attachment is expected to be eliminated. In this case one cell obtains a constitution not different from that of any non-crossover cell, while the other cell will receive the chromatid with two attachment points. This cell will lack completely the left end of the X chromosome and presumably would not give rise to a viable product. Thus, segregation **(z)** would not yield visible mosaic areas.

(2) Segregation **(x)**. In this case the two non-crossover chromatids segregate to opposite poles, while the chromatid with two attachment points becomes oriented parallel to the spindle axis in such a way that sister fibre points are directed toward opposite poles. The chromatid without any fibre point is again regarded as being left in the middle of the mitotic figure and thus eliminated. There are two ways by which such a process might result in two daughter nuclei.

First, it is possible that the double-fibre-point chromatid will not be included in the nuclei and so be eliminated. In this case cells with the following constitutions will be formed:

In experiment (1) twin cells with $y\ bb^{Df}$ and sn^3
" " (2) " " " $y\ sn^3bb^{Df}$ and $+$
" " (3) " " " $sn^3\ bb^{Df}$ and y
" " (4) " " " bb^{Df} and $y\ sn^3$

In all cases only one X chromosome will be present in each nucleus. The cells with bb^{Df} are not expected to survive. Thus single mosaic areas would be formed of the constitutions sn^3, $+$, y, or $y\ sn^3$. Actually a process of elimination cannot account for more than a very small number of spots, at best. For it does not account at all (a′) for the abnormally small size of setae in the majority of spots, (b′) for the $+^{sn}$ spots in experiment 1 (125 out of 209), (c′) for none except 1 spot (out of 37) in experiment 2, (d′) for the $+^{y}$ spots in experiment 3 (36 out of 70), (e′) for the not-$y\ sn^3$ spots in experiment 4 (9 out of 15), and (f) for the fact (table 25b) that out of a total of 22 spots in which the sex could be determined only 5 showed male coloration whereas the elimination process should lead to male spots only. The same arguments dispose also of the possibility that the two-fibre-point chromatid as a whole often becomes included in one of the daughter nuclei. In such cases the sister nucleus would have a 1X constitution as outlined above.

Let us therefore consider a second possibility. Again we regard the case that the two non-crossover chromatids go to opposite poles and that the two-fibre-point chromatid is arranged so that sister fibre points of the chromosome group segregate equationally. The two-fibre chromatid will then be subjected to conflicting forces, one end with its fibre attachment "pulling" in the opposite direction from the other. On the basis of the cytological observations of the authors named above, who studied two-fibre chromatids in meiosis we assume that the two-fibre chromatid breaks at some point under the stress and that the two fragments become included, each in one daughter nucleus. Such a hypothesis leaves room for two pairs of alternative possibilities resulting in four types of areas to be expected (fig. 10). The two-fibre chromatid will either contain sn^3 or $+^{sn}$ according to the region in which crossing over occurs in the inverted section, namely to the left of sn^3 (as measured in the non-inverted chromosome) or to the right of it. Also, the fragmentation break can fall on either side of the locus of sn^3. The consequences of these four possible occurrences are given in figure 10. Sister cells originate in each case which possess one complete X chromosome (disregarding the deficiency for bb) and one X fragment of varying length.

No prediction can be made as to the relative frequencies of the four processes. Crossing over to the left of sn^3 can occur in the section from locus 9–21 only, while crossing over to the right can occur between the loci 21 and 64. This fact would seem strongly in favor of crossing over to the right of the sn^3 locus. But as both the region to the left and to the right of sn^3 extends up to a fibre point in one of the two chromosomes the incidence of crossing over on the two sides of sn^3 might tend to become more equal. The presence of the bb^{Df} introduces another factor of unknown influence. With regard to the fragmentation break it may seem that it should preferably fall to the "right" of the sn^3 locus (measured in comparison to the non-inverted chromosome), the more so as the "right" section is not only so much longer than the "left" one but includes in addition to the active region the long inert part which on account of the *bb*-deficiency is absent on the other side of sn^3. However, no a priori reasoning should be applied to this situation because the effect of the inert material on the mechanical properties of the X chromosome is an open question since SCHULTZ's (1936) and OFFERMANN's (1936) discovery of visible cytological differences in X chromosomes lying in salivary gland nuclei with or without extra inert material.

If we want to compare our actual findings with the expectations from the proposed scheme we must call attention to one more point. All nuclei originating from the different processes possess unbalanced X-chromosomal constitutions. Each contains only once the normal left end of the X chromosome, from *y* to the beginning of the inversion, but each contains certain other regions in duplicated condition, once in the non-crossover chromatid and once in the chromosome fragment. The degree of unbalance is variable according to the breakage point of the fragmenting chromatid. *Zygotes* with such unbalanced constitution will, in most cases, not be able to give rise to viable individuals, but it is permissible to assume a certain degree of viability of *hypodermal areas*. This leads us to expect mosaic areas originating from these processes to be small in size and covered with setae of varying degrees of abnormal growth. In many cases it is to be anticipated that the viability of two sister cells is different so that only one will be able to give rise to a mosaic area. Three striking phenomena regarding table 25a are seen at once to be in agreement with such expectations, namely the facts (a) "relatively low number of spots with 2 or more setae in experiment 2 and 4," the fact (b) "relatively low number of twin spots in experiments 1 and 3," and the finding that the majority of setae were abnormally small. Furthermore the genetic type of spots found in each of the four experiments agrees with the expectations (fig. 10). Only very few spots occur which cannot be accounted for on the assumption of single crossing over within the inversion and fragmentation of the two-fibre-point chromatid; but these spots are to be expected from crossing over outside of the inversion and from multiple crossovers. It cannot be stated with certainty whether the four theoretical possibilities concerning the region of crossing over and of fragmentation are all realized. Experiment 2 indicates that the great majority of spots, the y sn^3 spots, originated as a result of one or all of the processes "crossing over to the left of sn^3, fragmentation break to the right of sn^3" **(l,r)**, "crossing over right, fragmentation left" **(r,l)**, and "crossing over right, fragmentation right" **(r,r)**. The *y* spots with small setae, which occurred in the same experiment, point to the occurrence of the fourth process "crossing over left, fragmentation left" **(l,l)**. In experiment 4 the presence of *y* spots seems to point to the occurrence of process **r,r**. If then we take the representation of processes **l,l** and **r,r** for granted, we have established the position of crossover and of fragmentation points to both sides of sn^3 in two combinations. There seems no objection to the assumption that the two other combinations, **l,r** and **r,l** are also realized. An attempt to evaluate the frequency of the four types on the basis of the actual data fails due to the variability of the results.

The total frequency of spots is higher in experiments 1 and 3, namely 10 per cent and 8 (or 6) per cent, respectively, as compared to experiments 2 and 4 where it is 4 and 2 per cent. This agrees in a general way with the fact (fig. 10) that in experiments 1 and 3, 7 out of the 8 segregational constitutions can potentially give rise to visible mosaic areas, while in experiments 1 and 4 only 4 out of the 8 constitutions can lead to visible spots.

Table 25b showed that 17 out of 22 spots in which the sex could be recognized were female and the remaining 5 were male. In these experiments both types of spots are to be expected, for a very short fragment in addition to the one X chromatid should result in a male spot and the presence of a longer fragment in a female spot.

Summary: The hypothesis of somatic crossing over in a heterozygous inversion accounts for the observed mosaic spots if it is assumed that the resulting chromatid without any fibre point is eliminated and the chromatid with two fibre points is fragmented so that each of the two fragments with its fibre point is included in a daughter nucleus.

Experiments involving y, sn^3*, and* bb^{Df}*; a Y chromosome present*

Females with essentially the same constitution in regard to X-chromosomal genes as those reported in the preceding chapter, but possessing a Y chromosome, were obtained and exhibited the spots summarized in tables 26a, b. A comparison of the two groups, without and with Y chromosome (table 27) yields three results:

(a) The presence of a Y chromosome increases the frequency of spotting. The percentages for the four pairs of experiments are:

TABLE 26a

Spots in experiments involving primarily y, sn^3 and bb^{Df}. A Y chromosome was present.

EXP.	CONST.*	IND.	SPOTS	%	sn^3			y			y-sn^3			y-sn^3		y-ysn^3	
				SPOTS	1	2	>2	1	2	>2	1	2	>2	2	>2	2	>2
1a	$y\ bb^{Df}/sn^3$ Y	161	62	—	3	1	3	36	12	7							
b		19	9	—	—	—	—	3	1	5							
					3	1	3	39	13	12							
Total		**180**	**71**	**39**	**7**			**64**									
2	$y\ sn^3\ bb^{Df}/+$Y	507	145	29	5(1?)	1	—	11(1?)	3	—	53	36	34	—		1	1
Total					**6**			**14**			**123**					**2**	
3	$sn^3\ bb^{Df}/y$ Y	458	57	12	34(3?)	1	2	10	2	5	—			2	1	—	
Total					**37**			**17**						**3**			
4	$bb^{Df}/y\ sn^3$ Y	863	50	6	10	3	3	12	5	10	4	—	3	—		—	
Total					**16**			**27**			**7**						

---

* All flies in exp. 1a contained an autosomal Minute. In exp. 1b and 4 the constitution in regard to the *bb*-locus was bb^{Df}/bb.

Experiment	*Without Y*	*With Y*
1	10	39
2	4	29
3	8 (or 6)	12
4	2	6

(b) The relative frequencies of different types of spots are changed by the presence of a Y chromosome.

(c) No phenotypes of spots in flies carrying a Y chromosome occur which did not also occur in flies without a Y chromosome.

A closer examination shows that the increase of total frequency of spots under the influence of a Y chromosome is mainly due to the rise of one particular class in each experiment. The classes with the heightened frequency are in experiment 1: *y*; in 2: $y\ sn^3$; in 3: sn^3. In experiment 4 two classes, sn^3 and *y*, have been increased.

TABLE 26b

Sexual coloration of critical spots from table 26a.

EXP.	sn^3	y	$y\ sn^3$		TOTAL	
	♂ COL.	NO ♂ COL.	NO ♂ COL.	♂ COL.	NO ♂ COL.	♂ COL.
1b	—	1*	--	—	1	0
2	—	---	27(1?)	1	27	1
3	1	1	—	—	1	1
4	—	6	1	1	7	1
Total					**36**	**3**

* This spot comprised a large part of the abdomen.

The data can be accounted for by the hypothesis that the presence of a Y chromosome leads to a higher frequency of the crossing over and fragmentation process **r,r** (fig. 10). This is obvious in experiments 1 and 2 where only *y* and $y\ sn^3$ spots will become more numerous. In experiment 3 where the process **r,r** results in *y* and sn^3 sister cells the further assumption is necessary that the viability of the *y* segregate is so much lower than that of the sn^3 segregate as to result mainly in sn^3 single spots. This assumption is compatible with the data in experiments 1 and 2. In experiment 4 the hypothesis leads to the expectation of an increase in + segregates which are not recognizable. According to this the higher frequency of sn^3 and *y* spots in 4 has to be accounted for in a different way. No explanation for this increase will be advanced as the rather low numbers would make any attempt unsafe. But it may be pointed out that apart from the very striking increase of the classes *y* and sn^3 in experiments 1 and 2, a slight increase is visible also in the percentage of some other spots.

The spots occurring in experiments with the Y chromosome are larger than in those without a Y chromosome (tables 25a and 26a). This is reflected especially in the fact that 70 out of 123 $y\ sn^3$ spots in experiment 1, table 26a, covered 2 or more setae. The significance of this result is doubtful.

The interpretation of the influence of a Y chromosome on somatic crossing over by means of a preferential increase in process **r,r** leaves still open the question as to the underlying mechanism. The occurrence of segregational products in connection with the process **r,r** is dependent on three conditions: (1) crossing over to the right of sn^3 and (2) fragmentation to the "right" of sn^3. Both conditions must be involved in causing the increase of the process **r,r**: higher frequency of condition 1 can be effective only if accompanied by higher frequency of 2. A rise of 2 by itself would not be compatible with the observed total increase of spots as it would mean only an increase in number of **r,r** processes at the cost of a decline of **r,l**. The result of these deductions is the hypothesis that the influence of a Y chromosome on the frequency of certain spots is accomplished by an increase in the frequency of somatic crossing over to the right of sn^3 (in the normal X chromosome) combined with an increase in fragmentation frequency in that part of the two-fibre-point chromatid which lies between sn^3 and the normal (not-bb^{Df}) end of the chromatid. A third element, a possible rise in the frequency of **x**-segregation at the expense of **z**-segregation can at best be only an additional factor. Further data regarding the influence of a Y chromosome will be discussed in following chapters.

TABLE 27

Summary of spots from experiments involving primarily y, sn^3, and bb^{Df}, without or with the presence of a Y chromosome. Details in tables 25, 26.

EXP.	CONST.	IND.	SPOTS	% SPOTS	sn^3	y	$y\ sn^3$	y-sn^3	y-$y\ sn^3$	♂ COL. +
1	$y\ bb^{Df}/sn^3$	2029	211	10	74	122	—	13	—	2
	$y\ bb^{Df}/sn^3$ Y	180	71	39	7	64	—	—	—	—
2	$y\ sn^3\ bb^{Df}/+$	838	37	4	2	12	21	—	1	1
	$y\ sn^3\ bb^{Df}/+$Y	507	145	29	6	14	123	—	2	—
3	$sn^3\ bb^{Df}/y$	872	70	8(6)	31	34	—	5	—	—
	$sn^3\ bb^{Df}/y$ Y	458	57	12	37	17	—	3	—	—
4	$bb^{Df}/y\ sn^3$	740	15	2	2	6	6	—	—	1
	$bb^{Df}/y\ sn^3$ Y	863	50	6	16	27	7	—	—	—

An exceptional case of segregation in experiments involving y, sn^3, bb^{Df} and an extra Y chromosome

All fourteen experiments recorded in tables 25a and 26a gave results which agreed among each other. A fifteenth experiment, however, had a fundamentally different outcome. The flies in this case were of the same constitution as those in "experiment 1 with Y," namely $y\ bb^{Df}/sn^3$; Y. They also contained the autosomal Minute 33j in heterozygous constitution, but so did many of the females of experiment 1a, table 26a, first line. Table 28 records the results which were obtained from a set of 7 cultures with two or three female and three male parents each. They were started several months apart from the experiments of table 26a.

TABLE 28

Spots from an exceptional experiment involving y, sn^3, bb^{Df} and a Y chromosome.

IND.	SPOTS	%	sn^3 2	y			y-sn^3 >2	y-sn^3 >2	SEX OF SPOTS (ALL y)*	
				1	2	>2			NO ♂ COL.	♂ COL.
146	277	191	1	98	59	115†	3	1(?)	--	--
—	—	—	1		272		3	1(?)	4	33‡

* In addition: 1 spot of the y-sn^3 twin spots showed ♀ coloration in the sn^3 part.

† 1 spot possibly belongs to the y-sn^3 twin spot class

‡ 1 spot which covered the 6th right tergite had ♀ coloration laterally but not toward the median.

The results deviate in three ways from those discussed in the last chapter: (a) 272 out of 277 spots belong to only one class; (b) the setae in spots were of normal size; (c) 33 out of 37 y spots were of male constitution.

It is unknown in which respect the flies in this experiment deviated fundamentally from those discussed above. That they contained a $y\ bb^{Df}$ and a sn^3 X chromosome as well as a Y chromosome is certain, both from the parental constitutions and from progeny tests of 10 individuals. Although it has not yet been possible to duplicate the results they have been presented here as the numbers involved leave no doubt as to their significance. Besides, it is possible to account for them by the following assumptions: if, for reasons unknown, pairing between the genetically active regions of the two X chromosomes was inhibited and if pairing and somatic crossing over between the homologous regions of the X and Y occurred, then only the sn^3 bearing X and the Y would be involved. This is because the bb^{Df} chromosome lacks the region which finds its homologue in the Y chromosome. Equational segregation of the three pairs of fibre points will result in a $y\ bb^{Df}$ chromatid going to each pole and in addition, in case of **x**-segregation of the crossover tetrad, in two sn^3 X chromatids going to one pole and two Y chromatids going to the other pole (fig. 11). The result will be 3X and 1X sister cells. The 3X cells and their descendants do not form visibly mosaic areas on account of their normal phenotype, while the 1X cells form *y* areas. The size of setae in these areas is normal, as the deficiency for *bb* in the X chromosome is "covered" by the Y chromosomes and no extra X chromosome fragment is present. The sex of *y* spots, if recognizable, will be male. It is obvious that the hypothesis fits most of the facts but it should be remembered that no independent tests of it are available at present. Spots other than *y* with male constitution may be thought to have originated in consequence of occasional crossing over between the X chromosomes as discussed in the foregoing chapter.

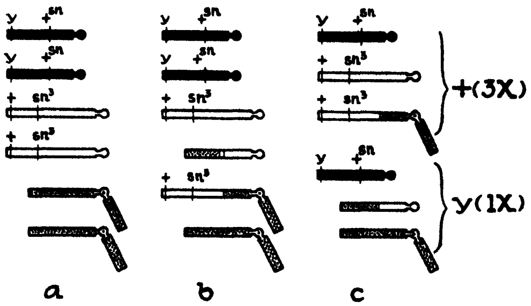

FIGURE 11. An hypothesis to account for the exceptional results in an experiment with y bb^{Df}/sn^3; Y flies. a. The six chromatids (2 bb^{Df}; 2 normal X; 2 Y). b. Four non-crossover chromatids and two chromatids resulting from crossing over between one normal X chromatid and one Y chromatid. c. The result of x-segregation of the six chromatids.

Experiments involving y, sn^3, bb^{Df} and Theta; no Y chromosome present

The hypothesis of the formation of a chromatid with two fibre points through crossing over within the inverted section, and the subsequent fragmentation of this chromatid, provided an explanation of the observed mosaic areas. It may, however, be asked if the differences in types of spots which occurred in the experiments with bb^{Df} as compared with those in which no inversion was involved were really great enough to warrant the elaborate scheme presented. Although the evidence presented seems to be in agreement with the hypothesis, further data will be adduced in the following sections which strengthen it greatly.

An analysis of the data given in table 29a, experiments 1 and 2 might profitably start with a comparison of the types and relative frequencies of spots in the experiments tabulated in table 13. There as here *y*, sn^3, and Theta were involved and the only difference consists in the presence or absence of the chromosome aberration connected with bb^{Df}. The results

TABLE 29a

Spots in experiments involving primarily y, sn^3, bb^{Df}, and θ, without or with a Y chromosome.

EXP.	CONSTITUTION	IND.	SPOTS	% SPOTS	sn^3			y			$y\ sn^3$			y-sn^3		+♂ COL.	OTHER SPOTS‡
					1	2	>2	1	2	>2	1	2	>2	2	>2		
1	$\frac{y \; + \; \theta}{y \; sn^3 \; bb^{Df}}$ no Y	392	702	180	98	81	203	102	30	22	11	5	9	18	95		
					382			154			25			113		13	15
1′	$\frac{y \; + \; \theta}{y \; sn^3 \; bb^{Df}}$ Y	19	72	379	11	10	21	7	4	1	1	1	2	—	9		
					42			12			4			9		—	5
2	$\frac{y \; + \; bb^{Df}}{y \; sn^3 \; \theta}$ no Y	262	53	20	2	—	—	33	8	6	—	—	—	—	—		
					2			47								4	—
2′	$\frac{y \; + \; bb^{Df}}{y \; sn^3 \; \theta}$ Y	203	167	82	1	2	4	60	32	48	—	—	—	1	1	18	—
					7			140						2			

‡ Exp. 1. sn^3-$y\ sn^3$ twin spots: 8. y-$y\ sn^3$ twin spots: 4. y-sn^3-$y\ sn^3$ triple spots: 3.
Exp. 1′. sn^3-$y\ sn^3$ twin spots: 2. y-$y\ sn^3$ twin spots: 2. y-sn^3-$y\ sn^3$ triple spots: 3.
Exp. 2. y-+♂ col. twin spots: 2.
Exp. 2′. y-+♂ col. twin spots: 4. y-sn^3-+♂ col. triple spot: 1.

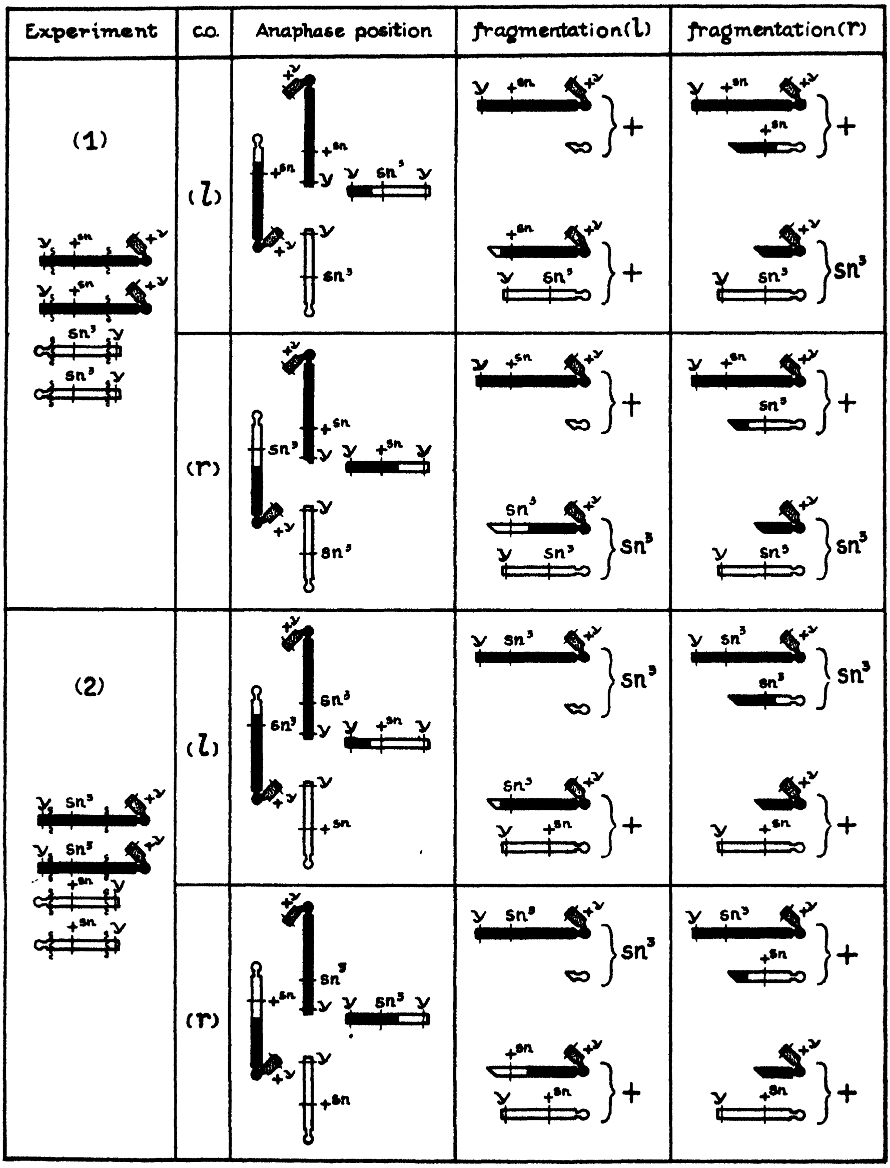

FIGURE 12. Two experiments involving y, sn^3, bb^{Df} and Theta (table 29a). The results of x-segregation after crossing over to the left (l) and to the right (r) of sn^3 and of fragmentation of the chromatid with two fibre points to the left (l) and to the right (r) of sn^3. The lines } indicate the end points of the inverted region.

are fundamentally different. Whereas the numbers of different kinds of spots calculated as percentages of total number of spots in:

Experiment (1) of table 13 were: 80 sn^3; 13y; 3 y-sn^3; 4 others, the percentages in:

Experiment (1) of table 29a were: 54 *sn*^{3}; 22 *y*; 16 *y-sn*^{3}; 8 others.

More striking still is the difference between the experiments 2 without or with *bb*^{Df}:

Experiment (2) table 13: 88 *sn*^{3}; 11*y*; 1 other.

Experiment (2) table 29a: 4 *sn*^{3}; 85 *y*; 11 others.

These differences become intelligible if one takes account of the nature of *bb*^{Df} as representing both a deficiency for the inert region of the X chromosome and an inversion for most of the genetically active region. If we make the same assumptions regarding crossing over within the inverted section, segregation, and fragmentation as those used in the chapter preceding the last we arrive at the expectations presented in figure 12. It is apparent that only *sn*^{3} spots will be produced (not considering at present that some of the + type segregates may lead to areas with male constitution). However, in experiment 1, 45 per cent of the spots were not-*sn*^{3} and it is certain that even a large proportion of the 54 per cent *sn*^{3} spots have to be regarded as rudiments of *y* and *sn*^{3} twin spots. In experiment 2 the number of *sn*^{3} spots deviates still more from expectation. Only 2 out of 49 significant spots were *sn*^{3}. Here the extreme rarity of *sn*^{3} spots does not necessarily indicate an equal rareness of crossing over within the inversion. If the most frequent process of crossing over and fragmentation is represented by the process **r,r,** as was the case in certain earlier experiments, then no *sn*^{3} spots will be produced by it in experiment 2, although they would appear in experiment 1 (fig. 12).

As crossing over within the inversion can at most account only for the occurrence of *sn*^{3} spots, other processes have to be looked for to furnish an explanation of the *y* single spots and *y* and *sn*^{3} twin spots. The small number of such spots recorded in table 13 had been shown to be due to somatic crossing over between the Theta-duplication and the homologous region of the right end (mainly) of the X chromosomes. If we consider the same process for the present experiments, we find the following expectations (fig. 13):

(a) Crossing over between Theta and an X chromatid of the chromosome not carrying *bb*^{Df} in experiment 1 leads to twin segregates with the constitutions *y* (3X)-*sn*^{3} (1X, 2 Theta); in experiment 2 to twin segregates *y* (3X)-+(1X, 2 Theta).

(b) Crossing over between Theta and a *bb*^{Df} carrying X chromatid with equational **x** segregation in experiment 1 leads to *y* (3X)-+(1X, 2 Theta) twin segregates and in experiment 2 to *y* (3X)-*sn*^{3} (1X, 2 Theta) twin segregates.

Thus crossing over involving Theta explains the occurrence of *y* spots. Will it also account for the *y-sn*^{3} twin spots in experiment 1? It will do so under the assumption that the viability of 1X, 2 Theta segregates is high enough to give rise to mosaic areas in a large number of cases. This assumption we had found justified during the analysis of experiments involving *y*, sn^3, *Mn*, and Theta. We can rule out the occurrence of crossing over process **b**, as it leads to *y*-sn^3 twin spots in experiment (2) which did not occur. This failure in crossing over between Theta and the right end of the bb^{Df} chromosome is in agreement with the probably complete absence of the homologous region in the deficient X chromosome. But if process **a** occurs in experiment 1 it accounts for the *y*-sn^3 twin spots, the *y* single spots (as rudiments of twin spots), and an unknown proportion of the sn^3 single spots (also as rudiments of twin spots). In experiment 2 it causes the *y* spots. It might be added that crossing over between the left end of Theta and the homologous left end of the X chromosomes yields *y* (2X) spots in both experiments and may contribute to this class.

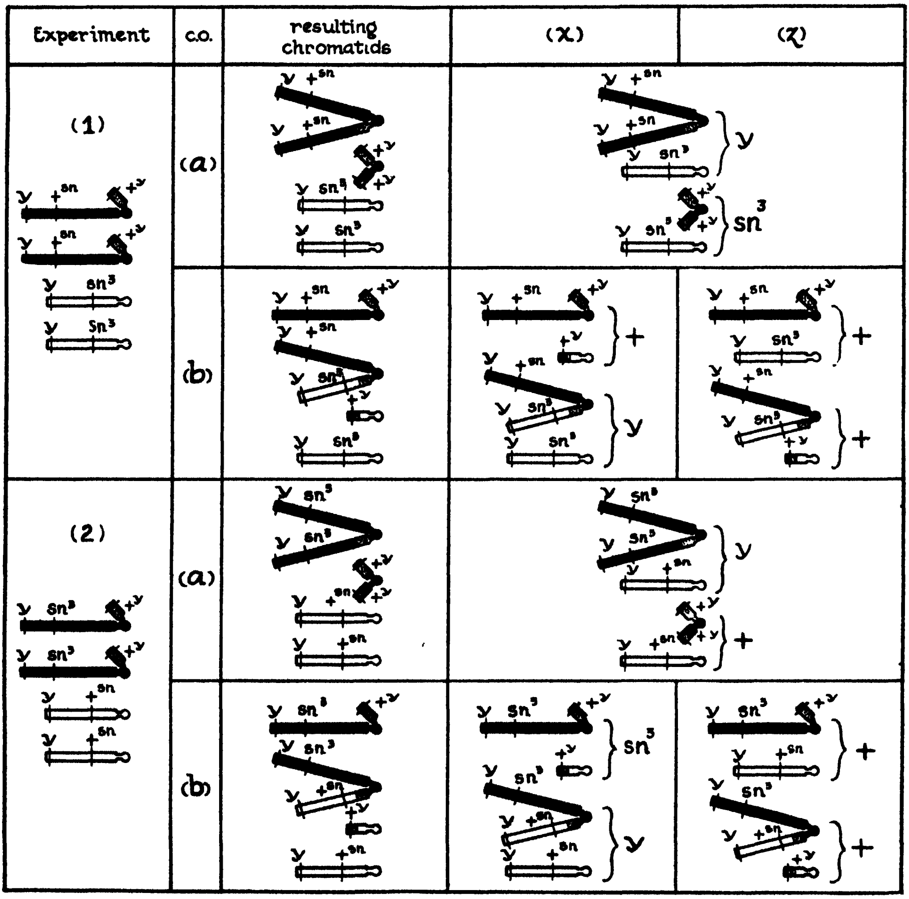

FIGURE 13. Two experiments involving *y*, sn^3, bb^{Df}, and Theta (table 29a). The results of crossing over between the homologous right regions of Theta and the X chromosomes and of subsequent **x**- and **z**-segregation.

An independent test of our explanation is provided by a consideration of the sex of spots (table 29b). In experiment 1, 35 sn^3 spots had no male coloration, 7 showed male coloration, and in 34 cases a small spot of male coloration occurred near to the sn^3 bristles. Singed-3 spots both with and without male coloration would be expected from crossing over within the inversion (fig. 12), according to the balance of sex factors brought about by the varying length of the duplicating fragment. In cases where crossing over within the inversion results in a sn^3, not-male-coloration segregate, the twin segregate will often have a constitution leading to $+^{sn}$, male-color areas. This may account for cases where a spot of male coloration was found near to sn^3, not-male-colored regions. (It is probable that some of these cases were sn^3 male-colored single spots in which the sn^3 trichogenic cells have become isolated from the rest of the cells constituting the spot.) Finally, those sn^3 spots which are rudiments of a y and sn^3 twin segregate produced after crossing over between Theta and an X chromatid ought to be of male coloration. It is probable that some of the male-colored sn^3 spots owe their origin to the last named process.

TABLE 29b

Sexual coloration of critical spots from table 29a.

NO. OF EXP IN TABLE 29a	sn^3		y		y-sn^3		+	y-
	NO ♂ COL.	♂ COL.	NO ♂ COL	♂ COL.	NO ♂ COL.	♂ COL.	♂ COL.	+♂ COL.
1	35	7; near 34	13	—	27	4; near 5	13	—
1′	3	—; near 2	1	—	3	—	—	—
2	—	–	6	—	—	—	4	2
2′	1(?)	—; near 1?	13	—	1	—	18	4

In addition exp 2′ 1 y-sn^3-+ ♀ col triple spot

All y spots in experiment 1 were of "no male color" (13 cases), in agreement with their expected 3X (or sometimes 2X) constitution. A discrepancy, however, exists in the cases of y-sn^3 twin spots. The hypothesis calls for y areas with not-male-coloration and sn^3 areas with male constitution. Four such cases were found. Five more cases, in which a small spot of male coloration occurred near the twin areas may in reality be also of the expected y ♀, sn^3 ♂ kind. But in the majority of critical y-sn^3 spots, (27 cases), no male coloration was visible in either twin area. The existence of this group raises doubts as to the validity of the present explanation. It is possible to make special assumptions to account for the unexpected type but they will not be given as proof is lacking.

The sex of spots in experiment 2 could be recognized in 8 y areas only. They were of not-male coloration in agreement with expectation and 2 were accompanied by an area of male coloration as demanded by segregation y (3X)-+(1X, 2 Theta).

No close analysis will be attempted for the observed single spots of normal setae with male coloration. Among other possibilities such spots are to be expected from crossing over within the inversion, and in experiment 2 as rudiments of twin spots from crossing over involving Theta.

If, in spite of the discrepancy pointed out, the general explanation given is accepted, then we come to the conclusion that the most frequent type of somatic crossing over is that between the right end of the Theta duplication and the homologous region of the not bb^{Df} bearing X chromatids. In the experiments of table 13 this process is rare but it is understandable that its relative frequency is high when bb^{Df} is present, for the following reasons. Normally the highest frequency of somatic crossing over occurs in the neighborhood of the fibre point. This region is abnormal in a bb^{Df} chromosome lacking most or all of its inert section as well as the bb locus and enclosing also one end point of the inversion. These conditions can be expected to interfere with pairing and crossing over of the right ends of the X chromosomes. But they should facilitate homologous pairing of the right end of the normal X chromosome with the homologous region of the Theta duplication.

Apart from the high relative frequency of certain types of spots the absolute frequency of mosaic areas is unusually high in comparison with the similar experiments of table 25a. It may be suggested that the presence of the Theta duplication is responsible for the difference. It contains inert material like a Y chromosome which, as shown above and again below, increases the total frequency of somatic crossing over.

No explanation can be given for the further difference between the frequencies in experiments 1 and 2 of table 29a.

Experiments involving y, sn^3, bb^{Df} and Theta; a Y chromosome present

The ability of a Y chromosome to increase the frequency of spots strongly is again demonstrated by a comparison in table 29a of experiments 1 and 2 with experiment 1′ and 2′. Under the influence of the Y chromosome the frequencies rise from 180 to 379 per cent and from 20 to 83 per cent. No change in relative frequencies of the different types of spots occurred nor did new types of mosaic areas appear. The coloration of the spots in regard to sex (table 29b) showed features similar to those brought out in the preceding section.

Apart from one discrepancy the experiments involving y, sn^3, bb^{Df}, and Theta with or without a Y chromosome seem to confirm the explanation proposed for similar experiments in which Theta was not present.

New facts were presented to show that somatic crossing over frequently

TABLE 30a

Spots in experiments involving primarily y, sn^3, Mn, bb^{Df}, and θ without or with a Y chromosome (see text).

EXP.	CONSTITUTION	IND.	SPOTS	%	sn^3			y			y sn^3			y-sn^3		y sn^3-sn^3	+	+♂ COL.	+♂ COL.-y		
					1	2	>2	1	2	>2	1	2	>2	2	>2	>2	>2		1	2	>2
1	$\frac{y\ +\ +\ Mn\,bb^{D}}{y\ w^{e}\ sn^{3}\ +\ \theta}$	2685	666	25	35	11	97	269	59	188	2	—	—	—	3	—	2	—	—		
						143*			516†			2		3							
2	$\frac{y\ w^{e}\ sn^{3}\ Mn\ bb^{Df}}{y\ +\ +\ +\ \theta}$	320	207	65	5	3	2	110	33	41	1	—	—	4	6	—	—	2	—		
						10			184			1		10							
3	$\frac{y\ +\ Mn\ \theta}{y\ sn^{3}\ +\ bb^{Df}}$	28	75	268	11	11	23	4	2	1	3	5	2	4	4	5	—	—	—		
						45			7			10		8							
4a	$\frac{y\ w^{e}\ sn^{3}\ Mn\ \theta}{y\ +\ +\ +\ bb^{Df}}$ no Y	143	80	56	—	—	—	34	16	18	—	—	—	—	—	—	—	10	1	—	1
									68											2	
b	$\frac{y\ w^{e}\ sn^{3}\ Mn\ \theta}{y\ +\ +\ +\ bb^{Df}}$ Y	223	502	225	—	—	1(?)	140	86	166	—	—	—	—	—	—	—	71	10	10	18
						1			392											38	
c	$\frac{y\ w^{e}\ sn^{3}\ Mn\ \theta}{y\ +\ +\ +\ bb^{f}}$ Y	151	202	134	—	—	—	63	40	54	—	—	—	—	—	—	—	33	4	2	6
									157											12	

---

* 51 of these were clearly $+^{M}$ and 9 *Mn*.

† 71 of these were clearly $+^{M}$ and 19 *Mn*.

involves the Theta duplication and that the constitution 1X, 2 Theta is viable in hypodermal spots.

Experiments involving y, sn^3, Mn, bb^{Df}, and Theta

The conclusions arrived at in the last section are once more found to be in accordance with a series of experiments which contained *Mn* in addition to the genes present in the last group. The results (table 30a) are in marked contrast to those of table 16a, where no bb^{Df} chromosome was involved; but they are of the kind to be expected from the theory developed in the last chapter. These experiments were partly made before the role of the Y chromosome in somatic crossing over was known. From independent evidence, however, it is most likely that no Y chromosomes were present in experiments 1 and 2 while an extra Y chromosome probably was present in experiment 4b. In experiments 3 and 4a it is certain that the females inspected had no Y chromosome and experiment 4c was devised in order that a Y chromosome should be present.

TABLE 30b

Sexual coloration of critical spots from Table 30a and from similar experiments.

EXP.	sn^3		y		$y\ sn^3$ NO ♂ COL.	$y\ sn^3$-sn^3 NO ♂ COL.	$+$♂ COL.	y-$+$♂ COL.
	NO ♂ COL.	♂ COL.	NO ♂ COL.	♂ COL.				
1	1	39‡	—	—	—	—	—	—
2	—	—	23	—	—	—	2	—
3	5	3	—	—	1(?)	1*	—	—
4a	—	—	5	—	—	—	10	2
b	—	—	7	—	—	—	71	38
c	—	—	10	15(?)	—	—	33	12
$\frac{+\ +\ Mn\ bb^{Df}}{y\ w^e\ +\ \theta}$	—	10	—	—	—	—	—	—
$\frac{y\ Mn\ \theta}{y\ +\ bb^{Df}}$Y	—	—	2	1(?)	—	—	—	4

‡ Determination of sex based on abdominal coloration in 26 spots; on wing length in 2 spots; wing length and sex comb in 1 spot; w^e coloration in 10 spots.

* Determination possible in the sn^3 spot only.

A short analysis on the basis of our previous findings follows (table 31):

(a) Single crossing over within the inversion and followed by fragmentation of the two-fibre-point chromatid leads to sn^3 $+^M$ and + areas in experiment 1, to + and sn^3 *Mn* areas in experiment 2, and to sn^3 *Mn* areas in experiment 3. No mosaic areas are produced in experiment 4.

(b) Crossing over between Theta and the homologous right arm of a not-bb^{Df} chromatid leads to the following twin segregates:

Experiment 1: *y*(3X)-*y* *Mn* bb^{Df} (1X)
Experiment 2: *y*(3X)-*y* sn^3 *Mn* bb^{Df} (1X)

Experiment 3: $y(M/M/+;3X)\text{-}sn^3$ (1X, 2 Theta)
Experiment 4: $y(M/M/+;3X)\text{-}+$ (1X, 2 Theta).

The 1X segregates of experiments 1 and 2 are expected to be inviable. Consequently *y* single areas will result in these two experiments. As hypodermal areas with three X chromosomes containing 2 Minute-n and one $+^{Mn}$ allele can be formed according to our earlier findings, *y* and sn^3 twin areas are expected in experiment 3 and *y* areas next to +-(1X)-areas in experiment 4.

TABLE 31

y, sn^3, Mn, bb^{Df}, θ

Types of segregates after different kinds of crossovers and fragmentation processes in experiments noted in table 30.

REGION OF C.O AND FRAGMENTATION	EXP. 1	EXP. 2	EXP. 3	EXP. 4
l,l	sn^3 Mn	+ +	dies Mn	dies Mn
l,r	sn^3 Mn	+ $sn^2\ Mn$	dies $sn^3\ Mn$	dies Mn
r,l	sn^3 Mn	+ $sn^3\ Mn$	dies $sn^3\ Mn$	dies Mn
r,r	+ Mn	+ $sn^3\ Mn$	dies $sn^3\ Mn$	dies Mn
c.o θ-X	$y(3X)$ dies	$y(3X)$ dies	$y(M/M/+)$ $sn^3/\underline{\theta\theta}$	$y(M/M/+)$ $+/\underline{\theta\theta}$

The actual findings lead to the conclusion that the majority of areas are derived from crossing over involving Theta. Although some of the following spots are probably produced by one or the other rarer processes, crossing over involving Theta can account for 516 out of 665 spots in experiment 1, for 184 out of 207 spots in experiment 2, for the *y* and sn^3 twin spots, and, as rudiments of twin spots, for the *y* single and an unknown number of sn^3 single spots in experiment 3, and in experiment 4a for all 80 spots namely the *y* and $+^{v}$ male-colored twin spots and as rudiments for the *y* single and $+^{v}$ male coloration single spots. The remaining spots can be derived from different processes of which single crossing over within the inversion accounts for the sn^3 spots in experiments 1 and 2 and for some of them in experiment 3.

In footnote ‡ to table 30a experiment 1 it is stated that of 90 *y* spots on head and thorax which were classifiable as to setae length, 71 were of approximately $+^{M}$ size and 19 were of M size. Crossing over between Theta and the right arm of the X chromosome leads to $y\ Mn/y+/y+$ (3X) spots which show about normal seta size. The *M* type spots therefore must have originated differently, for example, by crossing over between Theta and the homologous left part of the X chromosome. It is not possible to say whether the proportion of the two kinds of *y* spots in this head-thorax sample can or cannot be regarded as representative of the whole sample (see section on somatic segregation and ontogenetic pattern).

The determination of the sex of spots agrees on the whole with the expectation (table 30b). Attention may be directed to the fact that the sex of spots in experiment 1 could be judged not only by the coloration of abdominal spots but also by presence of sex combs, type of eosin eye color, and length of wing.

The absolute frequency of spots was high, even without the presence of a Y chromosome; 25 spots per hundred flies in experiment 1, 65 in experiment 2, 268 in experiment 3 and 56 in experiment 4a. Three of these values are, though somewhat higher, of the same order of magnitude as in experiment 2 of table 29a. The unusually high value of experiment 3 is similar to the one in experiment 1 of table 14. In both cases a $y\ sn^3\ bb^{Df}$ chromosome was present but the cause of the unusually high percentage of spots is not apparent.

The presence of a Y chromosome, in experiments 4b and c raises the total frequency of spots from 56 to 225 and 134 per cent. Besides, in experiment 4c a certain number of unexpected *y* spots with apparently male coloration were found. Some of these most probably belong to the *y* (3X)-$+^v$(1X) twin group; but others were of *y* male coloration indeed. Perhaps segregation similar to that found in the exceptional experiment with $y\ bb^{Df}/sn^3$; Y occurred here.

Information on experiments with $y\ Mn\ bb^{Df}/y\ w^e$ and $y\ Mn\ \theta/y\ bb^{Df}$ Y flies is added in table 30b. The results agree with the rest.

On the whole the theory of somatic crossing over in flies heterozygous for an X chromosome inversion has stood the exacting test provided by the experiments with *y*, sn^3, *Mn*, bb^{Df}, and Theta. If the theory is accepted, the present section adds weight to the deduction that two doses of *Mn* in triplo-X are not lethal to hypodermal areas. This will be of importance for the interpretation of mosaics in flies with a ring-shaped X chromosome (X^c).

Experiments involving the dl-49 Inversion

An X chromosome inversion has been involved also in certain experiments which were discussed earlier. This is the "dl-49" inversion (tables 5 and 13). It is a shorter inversion than the one associated with bb^{Df}, extending only from locus 11± to 42±. Consequently all crossovers from 42± to the right end are outside of the inversion; such crossovers formed the basis of the discussion. Crossing over within the inverted region leads to sn^3 spots and it is probable that some of the observed sn^3 spots owe their origin to such crossover types.

THE INFLUENCE OF A Y CHROMOSOME ON MOSAIC FORMATION IN FEMALES NOT CARRYING A bb^{Df} INVERSION

Only one experiment with flies containing two X chromosomes without an inversion gives information on the influence of a Y chromosome under these conditions. It concerns the flies of experiment 2, table 10 which have been discussed before. The female parents of these individuals were XXY so that they themselves consist of XX and XXY flies in about equal numbers. The total frequency of spots in these flies was 72.8 per cent as opposed to 3–12 per cent in experiments 1, 3, and 4 of the same group. The rise in frequency is presumably due to the presence of the Y chromosome in half of the flies. The influence of the Y is of course even greater in this experiment than the number 72.8 per cent indicates, for this represents the average percentage for all individuals of experiment 2, about one-half of which were of XX constitution.

In table 15 it had been shown that 9 out of 11 spots of experiments 1 and 4 of table 10 were not male-colored. However, 17 out of 30 spots in experiment 2 showed male coloration (table 32). According to the simplest form of the theory of somatic crossing over and segregation, no spots with male constitution were expected in these flies. The presence or absence of a Y was tested in 25 cases, the test consisting of a determination of the frequency of non-disjunctional sons and their fertility or sterility. Fertility indicates presence of a Y in the mother, sterility its absence. Lack of exceptional sons does not prove that the mother had no Y chromo-

TABLE 32

Sexual coloration of critical spots on y Mn/sn^3 *flies without or with a Y chromosome (cf table 10, experiment 2).*

TYPE OF SPOT	y			sn^3						y-sn^3		+♂ COL.	
	NO ♂ COL.	♂ COL. NEAR		NO ♂ COL.			♂ COL. NEAR		♂ COL.	NO ♂ COL.	♂ COL		
Number	2	3		9‡			5†		1*	2	1	7	
Y constitution of flies	?	?	XXY	XX	?	XXY	?	XXY	XXY	?	?	?	XXY
Number	2	1	2	1	7	1	1	4	1	2	1	5	2

Totals: not male-colored, 13; male colored, 17.

‡ Doubtful: 2 spots.

† Doubtful: 3 spots.

* Doubtful: 1 spot.

some but may only be due to too small a total progeny. This test method cannot serve as more than a first survey.

After disregarding the 5 individuals (representing 3 not-male-colored and 2 male-colored spots) which could not be tested there were left 10 not-male-colored and 15 male-colored spots. Only one of the 10 not-male-colored spots could be shown to have occurred on an XXY individual, while 9 out of the 15 male-colored spots were proved to have been on XXY females. The difference between these two distributions is significant ($\chi^2=6.25$; P between 0.01 and 0.02). We can conclude that the presence of a Y chromosome increases the number of spots of male coloration in females which do not carry an X chromosome inversion or duplication. The data are not conclusive as to the question whether male-colored spots in such females occur *only* if a Y chromosome is present, since the constitution of the 6 individuals with male-colored spots is not known. They may have been either XXY flies which did not produce exceptions or all or some of them may have been XX. In other experiments at least one case of a male-colored spot was found which occurred in a female in which certainly no Y chromosome was present. This female was supposedly perfectly normal in its chromosomal constitution, but the possible presence of a duplication of new origin cannot be excluded.

The action of the Y chromosome in the experiment just reported reminds us of the numerous male-colored spots in the exceptional experiment with $y\ bb^{Df}/sn^3$; Y females. There we demonstrated how somatic crossing over in XXY females between an X and the Y may give rise to XX and XXY twin spots, the latter constitution being responsible for the male coloration. It is possible that the occasional male spots found in several of our experiments (table 15, lines 1–3, and others) are due as a rule to the unsuspected presence of a Y chromosome.

If further experiments show that this is true, an at least partial explanation would be available for the fact that BRIDGES (1925) found the sex of spots in his *Mn* individuals to be always male, while most of our experiments with flies of similar constitution yielded mainly female spots. BRIDGES reports that the frequency of spots in his cultures was between 10 and 40 per cent. Considering that BRIDGES paid predominant attention to larger spots, these frequencies are very high. If we assume that supernumerary Y chromosomes were present in BRIDGES' stocks, we have an explanation both for the high frequencies of spots and for their male constitution.

MOSAICS IN FLIES HETEROZYGOUS FOR A RING-SHAPED X CHROMOSOME

Flies heterozygous for a ring-shaped "closed X chromosome" have been reported to have yielded numerous gynandromorphs (L. V. MORGAN,

TABLE 33a

Spots in X^c flies.

Experiments 2–4, 3, 9, 12–14 were inspected with low power only, therefore mainly head-thorax macrochaetae are involved.

EXP.	CONSTITUTION	IND.	SPOTS	sn^3 or f^5			y			$y\ sn^3$			+			y-sn^3 (or f^5)		OTHER SPOTS*
				1	2	>2	1	2	>2	1	2	>2	1	2	>2	2	>2	
1	$\frac{y\ X^c}{sn^3}$	153	147	100	12	19	11	2	2							—	1	
2	$\frac{y\ X^c}{sn^3}$	289	25	22	1	1	1	—	—									
3	$\frac{y\ X^c}{y\ sn^3\ bb}$	134	26				1	—	—	23	1	1						
4	$\frac{yX^c}{y\ sn^3\ bb}$ (XX & XXY)	483	59							58	—	1						
5	$\frac{y\ X^c}{w^{bf}f^5}$	—	109	67	9	5	21	—	3							2	1	1
6	$\frac{y\ X^c}{ysn^3\ \theta}$	97	97	71	6	10	5	—	—							2	3	
7	$\frac{y\ X^c}{(w)\ f\ Mn}$	45	47	—	—	1	—	—	3				14	2	26			1
8	$\frac{y\ X^c}{sn^3\ Mn}$	107	151	77	11	5	6	1	2				19	—	26	—	3	1
9	$\frac{y\ X^c}{ywMn\theta}$	27	15										7	—	8			
10	$\frac{y\ sn^3\ X^c}{+}$	—	27	4	—	—	3	—	1	14	1	3						1
11	$\frac{y\ sn^3\ X^c}{w\ bf\ f^5}$‡	110‡	57	11	3	11	2	—	1	17	1	6						5
12	$\frac{y\ sn^3\ X^c}{wmf\ Mn}$	58	35	1	—	—				1	—	3	20	2	6			2
13	$\frac{y\ sn^3\ X^c}{Bld\ w}$ (Bld-Minute)	—	20				6	—	2	8	2	2						
14	$\frac{y\ f\ Mn\ X^c}{sn^3}$	55	45	27	2	14							1	—	1			

1926, 1933). At a stage of this investigation when the relation of mosaic occurrence to somatic crossing over had not yet been recognized, varied experiments with this closed X chromosome, which hereafter will be referred to as X^c, were undertaken in order to clear up the problem of origin of mosaic areas. It was found that the frequency of gynandromorphs in flies heterozygous for X^c was not appreciably higher than usual. This had also been true in the later experiments of Mrs. MORGAN and it must be concluded that the original high incidence of gynandromorphs was not due to the closed X condition by itself, but due to either accessory or independent causes which have ceased to exist in the stock.

Although gynandromorphs were practically absent, a high frequency of spots of different kinds was encountered. The data are assembled in table 33a. In order to simplify the presentation, no distinction has been made as to the determination of the seta length in the different groups of spots. In most cases where only microchaetae were involved, no such determination was possible. However, when macrochaetae were included in spots it was seen that they fell into two groups, spots with about normal sized setae and spots with very small setae. A total of 219 spots could be thus classified according to bristle size. Often in cases of sn^3 or f^5 setae no finer distinction as to size was possible. Among the class $+^M$ it was found that generally the size of bristles was smaller than normal although distinctly larger than *Mn*.

In many cases there was a high correlation between the length of setae and the area covered by spots. Thus the total number of small and normal sized bristles in the sn^3 or f^5 group was 67 small and 4 normal among the single-setae spots, while among spots comprising more than one seta all 15 cases which could be classified as to seta size had setae of normal length. Among the *y* spots the relation is: 6 small and 8 normal single-bristle spots and 4 normal sized larger-area spots. Here no correlation is apparent for reasons which will become clear later. Among the *y* sn^3 spots were found: 105 single-seta spots with small and 2 with normal sized setae; larger spots—1 with small, 2 with normal sized setae.

It is immediately apparent that no simple hypothesis of elimination of a chromosome can explain the types of spots found. Such an hypothesis cannot account, among other facts, for the following:

(a) Appearance of numerous $+^M$ spots in experiments 7, 8, 9, and 12. If the *Mn* carrying chromosome were eliminated, *y* or sn^3 spots would be expected, if the not-Minute carrying chromosome were excluded, the remaining constitution would be lethal.

(b) Appearance of *y* and of sn^3 spots in experiments 10 and 11 where the original chromosomes carried both *y* and sn^3 or neither.

(c) The fact that 23 out of 55 critical spots were not of male constitution.

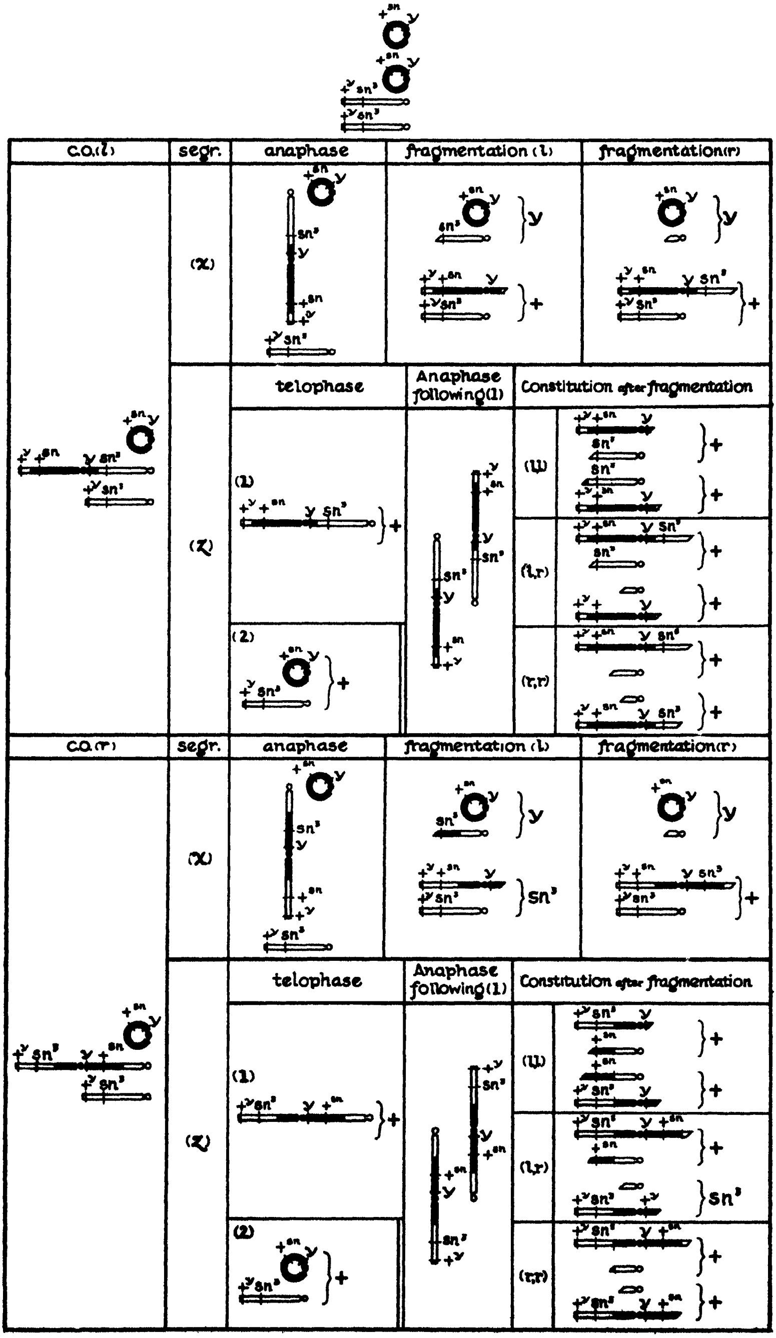

FIGURE 14. $y\ X^c/sn^3$. The results of crossing over to the left (l) and to the right (r) of sn^3. Subsequent x-segregation accompanied by different types of fragmentation of the two-fibre-point chromatid to the left (l) or right (r) of sn^3 or subsequent z-segregation followed, in one segregate, by different types of fragmentation (l, l; l, r; r, r) of both two-fibre-point chromatids.

(d) The appearance of abnormally short setae.

The occurrence of different types of spots and the relation between size of setae and amount of area covered by the spot become understandable under the assumption of somatic crossing over and segregation and taking account of the cytological peculiarity of the X^c chromosome, as will be shown now.

In examining the consequences of somatic crossing over we shall restrict ourselves to cases of single crossovers. The analysis will be given here in detail for one case only, represented by the experiments 1 and 2 and concerning flies with the constitution $y\ X^c/sn^3$ (fig. 14). Experiment 5 with $y\ X^c/w^{bf}\ f^5$ flies is very similar.

Crossing over between one strand of the ring chromosome and a strand of the normal X chromosome results in a long, open chromatid consisting of two full X chromatids with two fibre attachment points. This chromatid possesses one sister fibre point of the normal X chromosome on one end and one sister point of the ring chromosome in the middle. A double chromatid of this type has been called a "tandem" by L. V. MORGAN. If separation of sister points proceeds normally, two types of segregation have to be considered:

(1) **x**-segregation. The non-crossover chromatids go to opposite poles, the tandem being subjected to opposite forces on account of its two fibre points tending in different directions. This may result in either **(a)** non-inclusion of the tandem into the daughter nuclei and subsequent elimination, or in **(b)** fragmentation of the tandem between the two fibre points and inclusion of the two fragments within the two daughter nuclei. (A third possibility, the inclusion of the whole tandem chromosome into one daughter nucleus is least probable and will not be discussed.) Process **a** leads to *y* and sn^3 twin segregates with 1X constitution, process **b** to different pairs of segregates depending on the locus of crossing over and of fragmentation: **(l,l)**, **(l,r)** and **(r, r)** to *y* and + twins and **(r,l)** to *y* and sn^3 twins. While the segregates from **a** are both of male constitution, those of **b** contain a deficiency or a duplication for a left section of an X chromosome. Their sex is dependent on the extent of the deficiency or duplication.

(2) **z**-segregation. The non-crossover chromatids go to one pole, the tandem chromatid to the other pole. In this case the two segregates will be of the same genic constitution as the rest of the fly. However, the tandem-containing segregate will divide and its halves undergo fragmentation during the next cell division. The position of the two sister tandem-chromosomes is expected to be of such a nature as would result from sister fibre points going to different poles. Three combinations of fragmentation in the two separate tandems can occur: breaks in both to the

left of sn^3, in both to the right, and in one to the left and one to the right. Unless the two tandems happen to break at identical places unbalanced segregates will result. Their phenotype is in most cases + except in one in which a sn^3 segregate is produced.

When we compare these expectations with the actual results it becomes clear that the process **x** can at best account for only a small number of spots.

(1a) If the tandem were eliminated completely, *y* and sn^3 twin spots with male coloration should appear. Only one twin spot occurred; the proportion of *y* and sn^3 single spots is so unequal that only a few of them can be regarded as vestiges of twin spots. In addition, neither the fact that 11 out of 19 critical spots were not of male constitution nor the small size of most setae in single seta sn^3 spots agrees with the assumption of complete elimination.

(1b) Should the tandem undergo fragmentation during **x**-segregation we would meet a similar lack of agreement between facts and expectation. In three out of the four cases *y* spots will be produced, while *y* and sn^3 segregates result from the fourth. The observed *y* spots are regarded as results of **x**-segregation and fragmentation, but the great majority of spots are single sn^3 spots. Even if one assumes that the unbalanced *y* segregate of the twin cells is inviable, so that only single sn^3 areas are produced, the expectations as to bristle size would be mainly for normal length. For the sn^3 segregate would contain two complete X chromosomes and in addition a duplicating X fragment; such hyperploid condition leads to strong setae (PATTERSON, STONE and BEDICHEK 1935).

(2) The majority of spots can be understood when we assume **z**-segregation to have preceded them. The only segregate resulting in a visibly aberrant area is of the phenotype sn^3. Furthermore it contains only one complete X chromosome besides two fragments which together represent less than a second X chromosome. The sections represented by the two fragments are variable, depending on the locus of fragmentation. Such hypoploid conditions may be expected to result often in short-bristled hypodermal cells of low viability. The 122 sn^3 single-seta spots of experiments 1 and 2 and the 67 f^5 single-seta spots of experiment 5 correspond to this type.

To account for the smaller number of sn^3 spots which are of larger size and possess bristles of normal length one can assume both that some of them represent special hypoploid conditions due to **z**-segregation which are favorable in respect to genic balance and also that they are results of double crossing over to both sides of sn^3, a process which will lead to normal segregation of two and two X chromosomes and homozygosity for sn^3 in one segregate.

The sexual coloration of critical spots in experiments 1 and 5 was male in 9, not-male in 10 sn^3 spots and as regards y spots male in 1 and not-male in 2 cases (table 33b). No very definite expectation on the basis of the proposed theory is possible, since hypoploid conditions may lead to both types of sexual characteristics (which involve intersexual conditions) according to the length and region of the duplicating fragments involved. Those sn^3 spots which are derived from double crossovers are expected to be of normal female constitution.

TABLE 33b

Sexual coloration of critical spots from table 33a.

	sn^3 OR f^b		y		y sn^3		+♂	y-sn^3	y sn^3-f^b	y sn^3-y(?)
	NO ♂	♂	NO ♂	♂	NO ♂	♂		♂	♂	NO ♂
1	9	8	2	—						
5	1	1	—	1			1			
6	3	4						1†		
7				1*			1			
8	4	1						1		
10					1	—				
11		6				1			2	1
14	1	2								
	18	22	2	2	1	1	2	2	2	1

Totals: not male colored, **22**; male colored **31**.

† Determination of sexual coloration only possible in sn^3 area.

* Sex comb.

If one applies the theory of somatic crossing over, segregation, and fragmentation of the tandem chromosome to the other experiments reported in table 33, one arrives at specific expectations in each case with regard to the major types of spots. The derivations have to take account of the different regions of the chromosomes in which crossing over and fragmentation can occur and are rather lengthy in some cases. The resulting expectations are summarized in table 34 together with the observed facts. There is a good agreement both as to kind of main spots and as to the larger frequency of spots produced in consequence of **z**-segregation as opposed to **x**-segregation. One exception with respect to the latter point is found: In experiment 10 the number of y sn^3 spots exceeds that of sn^3 spots, although the former are expected from **x**- and the latter from **z**-segregation. Such a case probably would lose its peculiar character if one could adjust the expectations to the supposedly different frequencies of crossing over in different regions. But no attempt toward a finer analysis of the data presented will be made here. Such an analysis should include additional facts to be derived from the use of the newly discovered closed X chromosome which carries the normal allele of yellow.

TABLE 34

Expected and observed spots in experiments with X^c flies.

EXP.	CONSTITUTION	EXPECTATION‡		OBSERVED SPOTS	
		(z)	(x)	LARGEST CLASS	2ND LARGEST* CLASS
1, 2, 5	$\frac{y\ X^c}{sn^3 \text{ or } f^5}$	sn^3	y, y-sn^3	sn^3	y
3, 4	$\frac{y\ X^c}{y\ sn^3\ bb}$ (Y)	$y\ sn^3$	$y\ sn^3$	$y\ sn^3$	--
6	$\frac{y\ X^c}{y\ sn^3\ \theta}$	sn^3	sn^3	sn^3	—
7, 8	$\frac{y\ X^c}{sn^3\ Mn \text{ or } w\ f\ Mn}$	$+^M$, sn^3	y, y-sn^3	$+^M$	–
9	$\frac{y\ X^c}{y\ w\ Mn\ \theta}$	$+^M$	$+^M$	$+^M$	--
10	$\frac{y\ sn^3\ X^c}{+}$	sn^3	$y\ sn^3$, y	$y\ sn^3$	sn^3, y
11	$\frac{y\ sn^3\ X^c}{w^{bf}\ f^5}$	f^5, sn^3	$y\ sn^3$, y, $y\ sn^3$-f^5 etc.	f^5	$y\ sn^3$
12	$\frac{y\ sn^3\ X^c}{w\ m\ f\ Mn}$	$+^M$, sn^3	$y\ sn^3$, y	$+^M$	—
13	$\frac{y\ sn^3\ X^c}{\text{Bld}\ w}$ (Bld-Minute)	y, $y\ sn^3$	$y\ sn^3$, y	$y\ sn^3$	y
14	$\frac{y\ f\ Mn\ X^c}{sn^3}$	$+^M$, sn^3	$+^M$, sn^3, y-sn^3	sn^3	—

‡ Expectation in case of *y*, *sn* or *f* not specified in respect to *M* or $+^M$.

* Only given if larger than 10% of largest class.

There was an opportunity, in experiment 4, to discover a possible influence of the Y chromosome on the occurrence of spots. Part of these flies carried a Y chromosome, but no effect became apparent.

In addition to the demonstration that the theory of somatic crossing over can give an explanation for spots in flies with a closed X chromosome a new result is contained in these experiments. This is the relative frequency of **x**- and **z**-segregation. While in the experiments discussed in former sections of this paper **z**-segregation does not lead to mosaic areas and its frequency therefore cannot be directly determined, in the present cases it occurs far more often than **x**-segregation. It appears probable that this is a consequence of particular conditions brought about by the presence of the tandem chromosome. These seem to result in preferential chromatid segregation, so that the two non-crossover strands go most frequently together to one pole and the tandem with its two fibre points to the other pole.

Changes in ring-shaped chromosomes during somatic divisions have been demonstrated cytologically by McClintock (1932) in maize. She has pointed out that somatic crossing over is probably responsible for these changes and that they are correlated with the origin of mosaics.

SEX-LINKED MOSAIC AREAS IN SUPERFEMALES AND IN MALES

No special study of sex-linked spots in flies of not-female constitution was made but some incidental observations seem worth recording.

Superfemales ($3X+2A$)

(*1*) *The three X chromosomes free.* Of 11 superfemales of the constitution $y\ sl^2\ bb^{Df}/y\ sn^3bb/sn^3$ inspected, 3 were free from spots, while the remaining flies exhibited 18 *y* spots (1 seta: 12 spots; 2 setae: 3 spots; >2 setae: 3 spots). In five other flies of the same constitution 5 *y* spots and 1 *y* and sn^3 twin spot (2 setae) were found.

(*2*) *Two attached X chromosomes, one free*: 14 $\widehat{yy}/sn^3bb$ individuals possessed 3 *y* spots (1 seta: 2 spots; 2 setae (no ♂ col.): 1 spot); 19 $\widehat{yy}/y\ sn^3bb^{Df}$(Y?) individuals possessed 4 $y\ sn^3$ spots (1 seta: 1; 2 setae: 10; >2 setae: 2).

The interpretation of these spots according to the theory of somatic crossing over is obvious.

Males

Spots involving segregation of sex-linked genes generally cannot be discovered in males. The only possibility which should give visible mosaic areas would be a segregation of two sister X chromosomes into one nucleus and resulting in a female spot. No case of this kind was encountered but the available data are not extensive enough to exclude the occurrence of such spots.

The situation is different in case of presence of an X chromosome duplication. Males which, besides a $y\ sl^2\ bb^{Df}$ X chromosome and (most probably) a Y chromosome, possess a separate chromosome consisting of a Theta duplication attached to the short arm of a Y chromosome have not-yellow body color and setae. They show *y* spots with *y* setae very frequently. This would be understandable if somatic crossing over between homologous parts of the Theta chromosome and the Y chromosome and normal segregation occurs.

It should be added that 65 males of the constitution "$y\ sn^3$; $\overgroup{\text{Theta, Y}}{}^{\bullet}$; no free Y" did not exhibit spots; nor did 135 "$\overgroup{y,\ \text{T}}$heta; Y" and 85 "$y\ \overgroup{sn^3,\text{T}}$heta; Y" males.

RELATIONS BETWEEN SOMATIC CROSSING OVER AND THE ONTOGENETIC PATTERN

The frequency of somatic crossing over is dependent on different factors. An influence of the environment was shown by BRIDGES (MORGAN, STURTEVANT and BRIDGES 1029) who found a decrease in number of spots with the progressing age of the culture, independent of the age of the mother. Experiments in our laboratory demonstrate an effect of varying temperature (STERN and RENTSCHLER 1936). Genetic factors which influence the percentage of spots are the Minutes, an extra Y chromosome, and probably different other factors, as judged from the variability of our results.

The dependence of the frequency of spots on different agents results in the appearance of different types of mosaicism. We thus have a parallel to the variable frequencies of piebald areas in mammals or to mosaic conditions in many organisms in general. It is possible to carry the comparison further. It can be shown that not only the frequency but the size and distribution of spots over the body is variable and dependent on different agents. This means that the time and frequency of the origin of segregates is independently variable in different regions of the developing organism. That this is true for mosaics which have been ascribed to mutations of unstable genes is well known (DEMEREC 1935). The following gives a corresponding account for spots which are known to be due to somatic crossing over.

With regard to differences *in time* of occurrence we shall refer only to two earlier statements. It was found (1) that the proportion of "1-seta spots" to "larger than 1-seta spots" is different in different experiments (table 3; see also other tables) and (2) that left crossovers are represented by large and small spots in some experiments (table 8) while they result only in single-seta spots in others (tables 10, 12). The causes for the variability in time of origin of spots are not known for the experiments referred to under (1). As to (2), it seems that the presence of *Mn* is correlated with the shift in occurrence of double crossovers.

The dependence of incidence of somatic crossing over on the spatial ontogenetic pattern, that is, on the conditions offered by different body regions of the larvae will be illustrated by a series of results. Such a dependence first became evident from the analysis of table 3, where the

TABLE 35

Distribution, in percentage of total, of spots over head, thorax, and abdomen in different experiments.

CONSTITUTION	NO. OF SPOTS	HEAD	THORAX	ABDOMEN
$sn^3\ Mn/y\ g^2\ ty$	120	5	58	37
y/sn^3	212	4	43	53
$y\ Mn\ bb^{Df}/y\ w^e\ sn^3\ \theta$	666	4	36	60
$y\ w^e\ sn^3\ Mn\ \theta/y\ bb^{Df}$	80	1	3	96
$y\ w^e\ sn^3\ Mn\ \theta/y\ bb^{Df}$ Y	203	0	1	99
Setae Inspected*	—	4	30	66

* In this and in the following two tables the term "setae inspected" refers to the percentage of all inspected setae which were located on the respective body regions. For detailed account see "Methods" and table 1.

average increase of number of spots in Minute flies as compared with not-Minute flies was lower for the abdominal regions than for the head and thorax or from the fact that the proportion of single-seta spots to larger spots was lower in the abdomen than in the head-thorax region (table 3). Other examples of greatly varying distribution of spots over the three main body regions are given in table 35. While this table is concerned with total number of spots, a more detailed analysis can be derived from a separate consideration of different types of spots (table 36). In experiment 1 no different distribution of sn^3 single and y and sn^3 twin spots is expected, because nearly all sn^3 single spots are regarded to be rudiments of twin spots. This expectation is fulfilled, as the last columns indicate. With re-

TABLE 36

Distribution, in percentage, of different types of spots over head, thorax, and abdomen.

CONSTITUTION	SPOTS		HEAD	THORAX	ABDOMEN	χ^2	P
	TYPE	NO.					
(1) y/sn^3	y	59	10	48	42	y, twin: 9.614	<0.01
	sn^3	82	2	35	63	y, sn^3: 8.186	0.01–0.02
	y-sn^3 twin	67	0	40	60	sn^3, twin: 2.101	0.03–0.05
(2) $y\ bb^{Df}/sn^3$	y	109	0	16	84	y, sn^3: 2.203	0.01–0.02
	sn^3	60	0	8	92		
(3) $y\ sn^3\ bb^{Df}/y\ \theta$	y	151	0	8	92	y, sn^3: 23.321	<0.01
	sn^3	382	0	1	99	y, twin: 6.885	<0.01
	y-sn^3 twin	113	0	1	99	sn^3, twin: 0.020	> .99
(4) $y\ Mn\ bb^{Df}/y\ w^e\ sn^3\ \theta$	y	497	2	33	65	y, sn^3: 14.614	<0.01
	sn^3	134	8	34	57		
Setae Inspected		—	4	30	66		

spect to the distributions of y single and $y\ sn^3$ twin spots as well as for that of y and of sn^3 single spots, the χ^2 test points to significant differences. The majority of y spots represent rudiments of twin spots and should be distributed in the same way as the sn^3 single and the twin spots. Therefore the deviation is interpreted to mean that those y spots which owe their origin to crossing over between y and sn^3 have a distribution which is distinguished from the rest. The data indicate that the head and thorax regions are more favorable to left crossovers than to crossovers near the fibre point.

TABLE 37

Distribution, in percentage, of different spots over the abdominal tergites 1–7.

CONSTITUTION	SPOTS		ABDOMINAL TERGITES						X	P
	TYPE	NO.	1+2	3	4	5	6	7		
y/sn^3	y	24	4	25	21	29	21	0	y, sn^3: 1 403	0.8– 0.9
	sn^3	51	4	22	33	24	17	0	y, twin: 8 787	0.05–0.1
	y-sn^3 twin	40	10	25	33	17	15	0	sn^3, twin: 1.861	0.7 –0.8
$y\ bb^{Df}/sn^3$	y	55	5	13	32	41	9	0	y, sn^3: 16.390	<0 01
	sn^3	91	2	40	29	27	2	0		
$y\ sn^3\ bb^{Df}/y\ \theta$	y	139	19	18	22	30	11	0	y, sn^3: 20.590	<0.01
	sn^3	376	4	21	20	29	25	1	y, twin: 13.277	<0.01
	y-sn^3 twin	112	4	22	20	32	22	0	sn^3, twin: 0.8417	0.5–0.7
$y\ Mn\ bb^{Df}/y\ w^e\ sn^3\ \theta$	y	323	13	30	32	21	4	0	y, sn^3 35.0392	<0.01
	sn^3	77	5	23	21	30	20	1		
Setae Inspected		—	10	13	17	24	24	12		

No significantly different distribution of the two types of spots is apparent in experiment 2. Both types result mainly from single crossovers within the inversion. An analysis is impeded by the possibility that different body regions may conceivably be variable in their effect on the viability of hypo- and hyperploid cells and thus lead to a differential survival of spots. This factor may play a role also in the other experiments to be discussed.

In experiment 3 no significant deviation of the distribution of sn^3 single and twin spots occurred, although these spots owe their origin largely to different processes. However, the distribution of y spots differs from both that of sn^3 and that of twin spots. While the first deviation is not surprising, since the two kinds of spots are mainly results of different crossovers, the distribution of y single and of twin spots should be identical, for the y spots are regarded to be rudiments of twin segregates. Possibly viability differences of the y and sn^3 twin segregates in different body regions are responible for the result.

In experiment 4 the distributions again differ significantly. Dependence of the occurrence of the two kinds of crossovers or of the viability of segregates upon conditions in different body regions seem to be involved.

The same four experiments which were considered with regard to head-thorax-abdomen distribution of spots have been analyzed as to location of spots on the tergites of different abdominal segments (table 37). In experiment 1 no significant deviation in distribution occurred between *y* and sn^3 single spots nor between sn^3 single and twin spots, while the *y* single and the twin spots were probably significantly different. Taking account of the smaller numbers, the earlier comments on this experiment seem to apply here likewise. In experiment 2 the *y* and sn^3 spots are doubtless distributed differently on the abdomen. In experiment 3 the similarities and differences resemble those of the distributions over head-thorax and abdomen. Lastly, in experiment 4, a striking dependence of type of spot on the abdominal region is apparent, showing a peak in frequency for *y* spots on the third and fourth segments and for the sn^3 spots on the fifth segment.

TABLE 38a, b

Distribution of y and sn^3 *spots over different body regions. Only spots from* $yMnbb^{Df}/yw\ sn^3\ \theta$ *flies of tables 36, 37 are included which occurred on individuals bearing at least one of each kind of spot.*

38a

SPOTS		HEAD	THORAX	ABDOMEN
TYPE	NO.			
y	29	2	6	21
sn^3	37	2	16	19

38b

NO.	ABDOMINAL TERGITES				
	1+2	3	4	5	6
21	4	7	4	4	2
19	1	5	3	6	4

It might be asked whether the differential distribution of different kinds of spots may be due to their occurrence on different individuals. To answer this question tables 38a and b are presented, in which are included all those *y* and sn^3 spots of experiment 4 which occurred on individuals bearing at least one of each type. The numbers are too small to give significantly different distributions. However, in the abdominal series it is evident that the trend of frequencies for the *y* and sn^3 spots coincides well with those of table 35, experiment 4.

The interpretation of the observed differences in abdominal distribution has to take account again of both differences in incidence of crossovers and of viability values. But it seems improbable that the survival values of different segregates vary enough in different tergites to cause the observed patterns by themselves. The participation of the body pattern as differential in regard to occurrence of somatic crossover types is regarded to be a contributing if not the main factor.

A comparison of the observed distribution of frequencies of spots with the numbers of setae inspected in different body regions (see last line of tables 35–37) shows that no random distribution took place. This is a further demonstration of the influence of body pattern on crossover occurrence. A finer analysis will depend on detailed knowledge and comparison of the developmental events within the different imaginal discs.

DISCUSSION

The evidence presented in this paper shows that mosaic areas on the body of *Drosophila melanogaster* appear in a varying percentage of flies whenever they are heterozygous for genes whose homozygous effect is recognizable in small spots. Theories as to the causation of these spots have to be based on somewhat intricate deductions. The observable phenomenon is limited mainly to yellow or singed single spots, or yellow and singed twin spots, in different proportions and with a few other attributes such as sexual coloration or seta length. The test of any theory as to the mechanism of spot production has to consist in its application to a varied group of genic combinations. Such a test is able to exclude definitely theories which cannot account for the actual facts. But if it succeeds in giving a satisfactory basis for them, it cannot claim a final "proof." In our special case we can say that the theory of chromosome elimination has been shown definitely unsatisfactory. The validity of the theory of somatic crossing over and segregation rests on its faculty to explain the manifold results presented in this paper. Throughout the text the word "assumption" has been used freely in order to leave no doubt as to the procedure of deduction. But it should be noted that most of these assumptions are justified from other experience.

There is a higher degree of safety in the discussion of somatic crossing over in cases where the X chromosomes were normal than in those where inversions or the closed X chromosome were involved. In these latter cases the claim is made that the proposed theory is able to account for most facts (some notable discrepancies have been pointed out), but the possibility of inventing other schemes should be stressed.

This analysis of somatic crossing over can by no means be regarded as complete. It was restricted in most cases to a consideration of single crossovers, although the rarer occurrence of double crossovers has been ascertained. No attempt was made to discuss multiple crossing over in detail, as for instance the relation of the different crossovers to different chromatids, or questions of interference. Neither has the probability been dealt with that somatic crossover processes may occasionally occur consecutively for two or more cell generations. Such repeated crossovers are thought responsible for the occasional triple spots as well as for certain twin spots. A filling out of these gaps in our knowledge although desirable is perhaps not likely to lead to results of general significance. But this might be different with respect to other problems raised in the course of study. To name some of these: what is the "physiological Minute condition" which is responsible for somatic crossing over? Why is there correlation between the locus of Minutes and the region of crossing over induction, between sex-linked Minutes and crossing over in the X chromosome, between autosomal Minutes and crossing over in the autosome, between locus of Minute to the left or to the right of the fibre point and crossing over in the corresponding arm? What is the mechanism of the Y chromosome effect on crossing over? What is the explanation for the exceptional, but self-consistent experiment with $y\ bb^{Df}/sn^3$; Y females?

The mechanism of mosaic formation rests *primarily* on the occurrence of somatic crossing over. If the mechanism of fibre point separation in mitosis remains undisturbed, as has been shown to be true, then genic segregation is a *necessary consequence* of crossing over. There is thus no need to assume two separate processes (1) somatic crossing over and (2) somatic segregation.

Abnormalities in chromosome behavior as causes of mosaic formation have been recognized before. Elimination of chromosomes or non-disjunction has been the most general explanation, for example, in gynandromorphs of Drosophila (MORGAN and BRIDGES 1919), or in certain types of endosperm mosaics in corn (EMERSON 1921, 1924). The idea of possible somatic crossing over was introduced by SEREBROVSKY (1925) in an interesting paper on the appearance of aberrant feathers on chickens of different genetic constitutions. SEREBROVSKY sums up his findings by stating "crossing over cannot be either proved, or denied" and he did not recognize it as the *cause* of somatic segregation. A different interpretation of his cases was consequently given by P. HERTWIG and RITTERSHAUS (1929) and by the present author (1928a, 1933). But it must be said now that SEREBROVSKY probably came nearer to the right explanation than his critics, although certain difficulties remain.

Actual segregation of the chromosomes among somatic cells was genetically demonstrated first by PATTERSON (1929a, b; see also FRIESEN 1935) who subjected Drosophila larvae to X-rays and obtained twin spots, besides many single spots. The author "cannot state definitely the nature of the mechanism which produces the segregation. It almost certainly involves some form of synapsis . . . in the somatic cells." On the basis of our present knowledge we believe the mechanism to have been somatic crossing over as suggested earlier by PAINTER (1934). And we are inclined to regard most cases of mosaic formation in X-rayed larvae not as results of elimination of parts of chromosomes as assumed before (Patterson 1930) but as indication that X-rays can induce crossing over in somatic cells.

There are some cytological findings which bear on this discussion. McClintock (1932) obtained cytological evidence of different chromosome constitution in diverse cells of a variegated corn plant. Her finding of different degrees of increase or decrease in length of a ring-shaped chromosome in different cells pointed strongly to some kind of somatic crossing over.

In *Drosophila melanogaster* Kaufmann (1934) could show the occasional presence of chiasmatype-like configurations between homologous chromosomes in somatic cells, before genetic evidence for somatic crossing over was available. And Peto has recently shown (1935) that chiasmata are formed in cells of root tips of *Hordeum vulgare* under the influence of radiation, a cytological correlate to Patterson's experiments.

How frequently mosaic production is to be regarded as caused by somatic crossing over can be determined by new experiments only. In *Drosophila melanogaster* it seems by far the most prevalent mechanism. In all our experiments in which products of elimination or non-disjunction could be distinguished from those of somatic crossing over and segregation the majority of spots was understandable only by assuming the latter process, whereas the small minority could be explained on either hypothesis. This was true both for the smaller spots and for the occasional larger spots comprising more than one imaginal disk. There is one exception to this statement: the gynandromorphs described by Morgan and Bridges (1919) and other workers cannot be explained by crossing over and segregation. This is perhaps significant. The somatic pairing of homologous chromosomes which is typical in Diptera becomes visible first in the prophase of the second cleavage division (Huettner 1924). As most gynandromorphic conditions originate during the first division a causal connection becomes probable.

One might suspect that the somatic chromosome pairing in Drosophila would facilitate the occurrence of crossing over. This would point to crossing over as a cause of mosaic formation in Diptera mainly. But as we do not know the reason why certain cells do undergo crossing over we are hardly justified at present in drawing this conclusion. On the other hand we should hold open the possibility of non-homologous somatic crossing over as it seems to occur in corn (McClintock 1932, Jones 1936). In Drosophila no evidence for non-homologous crossing over is available.

Many cases of inherited mosaic formation have been described as consequences of mutations of "unstable" genes (Demerec 1935). The present writer (1935) has proposed a hypothesis according to which the behavior of unstable loci should be regarded not as an internal change in a gene but as a result of "mechanical" changes at the locus, brought about by somatic crossing over. A model for such unstable loci was elaborated. Recently SCHULTZ (1936) has proposed a different cytological configuration in cases of unstable loci, which, being based on direct observation, is superior to the original speculation although probably not final either. An essential part of SCHULTZ's scheme is again the assumption that somatic crossing over changes the cytological configuration and thus leads to mosaics.

The finding of somatic crossing over throws some light on the mechanism of chromosome separation in mitosis. Normally the passing to different poles of chromosome halves implies a separation of whole sister strands. In cases of crossing over separation may occur in the normal way for one particular point only while the rest of two sister chromosomes may pass to the same pole. This particular point is the fibre attachment locus. We have here a genetic demonstration of its role in mitosis.

That the occurrence of crossing over without reduction of chromosomes is of significance for theories of meiosis and mitosis needs hardly to be pointed out.

The value of somatic segregation as a tool for the analysis of gene action is obvious. The process has been successfully used by DEMEREC (1934 and later) in his studies of the action of small deficiencies in hypodermal segregates (cf. also STERN 1935). A few more facts have come to light in the foregoing pages: viability of hypodermal areas containing one X chromosome and two Theta duplications; or one X chromosome and one long X duplication of varying length; or two X chromosomes and similar duplications; or three X chromosomes containing two Minute-n loci. All these constitutions are lethal to zygotes. The genic unbalance represented by them is thus not able to sustain full ontogenetic development but is compatible with division and differentiation of cells of imaginal discs.

SUMMARY

(1) Mosaic areas on the body of *Drosophila melanogaster* appear on flies which are heterozygous for genes whose homozygous effect can be recognized in a small spot.

✓(2) The frequency of spots is increased by the presence of Minute factors.

✓(3) Spots of sex-linked characters occur with higher frequency when either sex-linked or autosomal Minutes are present, but sex-linked Minutes are more powerful than autosomal ones. Autosomal spots are more frequent in the case of presence of autosomal Minutes than of sex-linked

Minutes. Different Minutes show different degrees of ability to induce spot formation.

(4) The mechanism of mosaic formation is not based on simple elimination of chromosomes but on processes of somatic crossing over involving two strands of a four strand group. Segregation of the four strands occurs in an equational, typically mitotic mode in respect to the fibre points. It leads to homozygosis of originally heterozygous genes. No reduction of number of chromatids takes place in normal cases. These conclusions are derived from an analysis of types and frequencies of twin and single spots and of the number of X chromosomes present as judged by the secondary sexual characters of favorable spots. Interpretations based on experiments with certain combinations of genes have been verified by tests of validity in other combinations of the same genes.

(5) The increase of frequency of sex-linked spots is not directly dependent on the localized, material constitution of the chromosomes involved in somatic crossing over but rather on the general "phenotypic Minute reaction" in development.

✓(6) The relative frequencies of somatic crossovers in different regions of the X chromosomes are different from those of germinal crossovers. Somatic crossing over is more frequent near the fibre point. The presence of Minute-n accentuates this shift.

(7) The X chromosome duplication "Theta" frequently undergoes somatic crossing over with the X chromosome—more frequently in the homologous right than in the homologous left regions. Germinal crossing over involving Theta is very rare.

(8) Somatic crossing over involving Theta followed by equational segregation leads to twin segregates of the constitution 3X chromosomes-1X chromosome.

(9) The apparently exceptional behavior of the bobbed character, which does not become visible in spots, is understandable under the assumption that no somatic crossing over occurs to the right of the bobbed locus.✓

(10) Somatic crossing over involving the sex chromosome occurs in superfemales and in males.

✓(11) Somatic autosomal crossing over takes place in both sexes, though more frequently in females. A peculiar specificity of the Minute effect leads to crossovers in that arm of the third chromosome in which the Minute itself is located. Most crossovers are concentrated near the fibre point region.

(12) Somatic crossing over between X chromosomes heterozygous for the bb^{Df} inversion occurs within the inversion. It leads to a chromatid

which possesses no fibre point and is thus eliminated, and to a complementary chromatid with two fiber points. This chromatid becomes fragmented and each fragment is included in a daughter nucleus.

(13) When Theta is present in cells heterozygous for the bb^{Df} inversion the most frequent type of somatic crossover involves Theta and the not-inverted, not-bb^{Df} chromosome. (A discrepancy is pointed out between this interpretation and the observed facts.)

(14) The presence of an extra Y chromosome in flies discussed under (12) increases the frequency of somatic crossing over within the inversion to the right of sn^3 as well as the frequency of fragmentation of the two-fibre-point chromatid, also to the right of sn^3.

(15) In flies discussed under (13) the presence of an extra Y chromosome increases the frequency of crossovers involving Theta.

(16) An exceptional series of cultures with XXY females gave results which can be interpreted as caused by somatic crossing over between X and Y chromosomes, leading to XXX and X segregates.

(17) Somatic crossing over in flies heterozygous for a ring-shaped X chromosome leads to a two-fibre-point "tandem" chromatid. Segregation occurs preferentially so that the two non-crossover chromatids go to one pole and the tandem chromatid to the other. In the following division the tandem chromatid becomes fragmented.

(18) In different experiments certain segregated constitutions are not sufficiently viable to give rise to mosaic areas. Others, though not viable as zygotic constitutions, permit the formation of hypodermal spots.

(19) Under different genetic conditions different patterns of mosaics are formed. The proportion of small to larger spots can vary. In Minute-n flies crossovers to the left of *Mn* occur later in development than crossovers to the right. Various genetic constitutions have differential effects on frequency and size of spots in various body regions. Different types of spots in flies of the same constitution are differently distributed over the head, thorax and abdomen or over the different abdominal segments.

(20) In the discussion, a short survey is given with reference to mosaic formation in general and its relation to somatic segregation.

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GENES FROM RADIUM TREATMENT AFFECTING POLLEN-TUBE GROWTH IN DATURA[1]

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INTRODUCTION

GENES affecting pollen-tube growth in *Datura stramonium* come under two general categories: (1) those which may be transmitted through both the eggs and the pollen, with recognizable abnormalities in the growth of the pollen tubes, and (2) those which are not transmitted through the pollen, but are transmitted maternally. The first category is illustrated by the gene for tricarpel (*tc*) (BUCHHOLZ and BLAKESLEE 1927). Plants homozygous for tricarpel are carried in our stock cultures and maintain themselves as pure lines, while types of the second category must be carried as heterozygotes.

The present paper has to do with the second category of pollen-tube genes. They comprise the genes giving pollen-tube growth distributions of the types V, VIa, VII, and VIIb, which were briefly described in a recent account (BUCHHOLZ and BLAKESLEE 1932a). In an earlier description of some of these gene types (BUCHHOLZ and BLAKESLEE 1930) we called them "lethals of pollen-tube growth," although they might better be spoken of as pollen-tube genes. It should be remembered that a gene of this kind is lethal only to the male gametophyte which carries it. In consequence, due to segregation in the reduction divisions, half of the pollen tubes from a heterozygous parent show the character and half are normal. These types may be recognized through growth tests of the pollen of the plants which are heterozygous for them, the so-called carrier plants.

Nothing may be found in the external morphology of the carrier plants which would distinguish them from non-carriers. The only direct tests which positively distinguish carriers from non-carriers are tests of the pollen when grown on the stigma and in the style of a test flower. For many of these genes any flower of a Datura may serve as a test flower, though the pistils of standard Line 1 or self pollinations are used in our routine work (BUCHHOLZ 1931 and BUCHHOLZ and BLAKESLEE 1932a).

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From among more than 40 genes affecting pollen-tube growth, which had been recognized up to 1931 and tested for their egg-transmission, we selected for special study five which belong to the second category. We have symbolized these: *s-1*, *s-2*, *sb-1*, *sb-2*, and *lp*. These carrier strains may be continued by identifying and selfing a carrier plant in each generation. Our practice, however, is to keep these genes in our standard Line 1 race by each generation pollinating some of the carriers with the pollen of Line 1. These genes are now in the 4th to 6th generation of backcrossing to Line 1 and, counting two selfed generations, they are from five to seven generations removed from the carrier in which they were originally found.

All of these pollen-tube genes were obtained in the progeny of irradiated plants. Most of them came from plants in which the parent pollen had been subjected to radium treatment. Their presence could be recognized by abnormalities in pollen-tube growth whenever the pollen of a carrier plant was tested, though proof of their egg-transmissibility required tests of their offspring. The five genes selected were chosen because of their variety as pollen-tube abnormalities and because of the distinctive character represented by each type of abnormality (BUCHHOLZ and BLAKESLEE 1932a) and the consequent ease and certainty in their diagnosis.

Genes affecting pollen-tube growth do not necessarily come from the treatment of pollen. The gene *s-1* was not derived from pollen treatment, but from the treatment of an ovary 36 hours after pollination (12–14 hours after fertilization). The change represented by this gene was therefore induced in the zygote, probably before the fertilized egg had completed its first mitosis. We have also observed pollen-tube mutations in the progeny of treated pollen tubes (BUCHHOLZ and BLAKESLEE 1930), in the progeny of seeds treated with X-rays, and in plants resulting from the treatment of flowers after fertilization. It may be recalled that tricarpel (*tc*) came from the progeny of a haploid plant, so that the origin of this gene was not in any way connected with radiation (BUCHHOLZ and BLAKESLEE 1927).

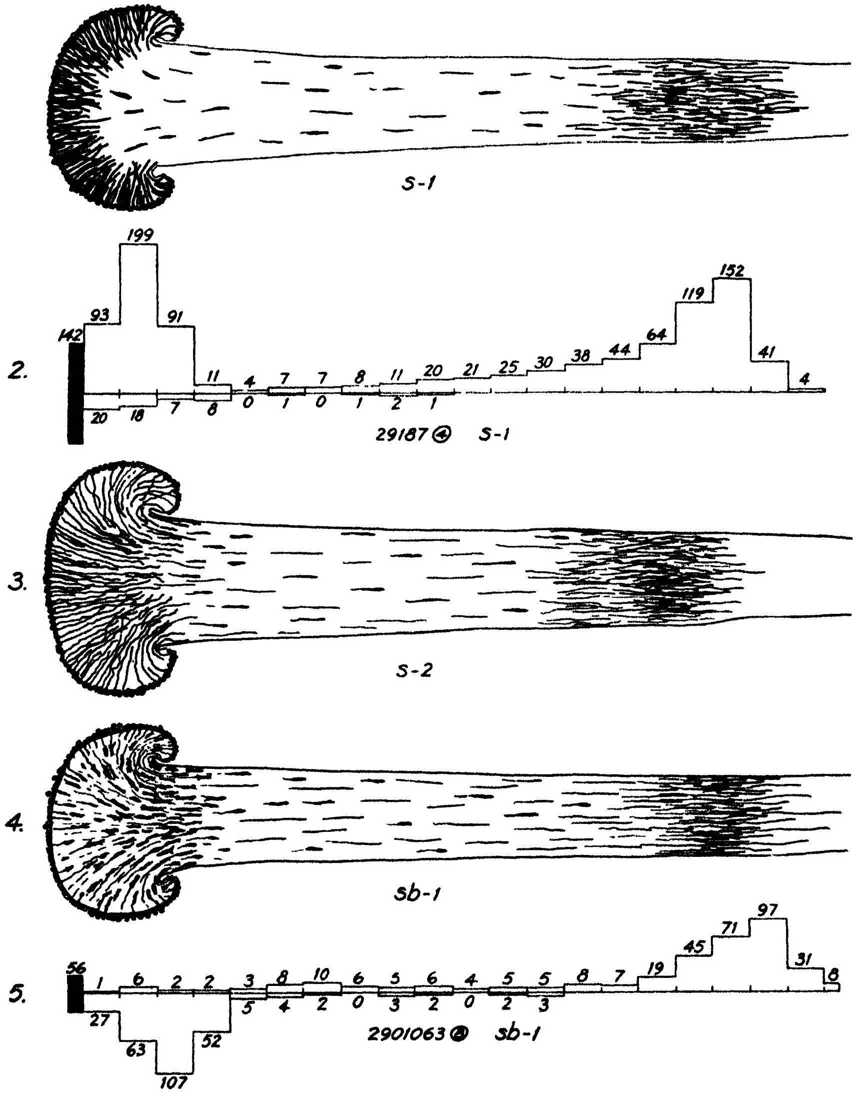
Our purpose in the rather intensive study of these pollen-tube genes was to establish their mode of inheritance and to discover their location on particular chromosomes. It is likely that genes of this kind may have an important role as a type of lethal in plants and their better appreciation may serve to throw much light on the nature of certain kinds of sterility and, more particularly, on the role of this mechanism of gametophytic selection in the evolution of plants.

DESCRIPTION OF THE POLLEN-TUBE TYPES

The gene *s-1* (short pollen tube) was the first which we used extensively in crosses in an effort to discover linkages. In a carrier plant it is a recessive so far as the effect on the sporophyte is concerned. All carriers are fully as vigorous as non-carriers, and the pollen of a plant which carries this gene is perfectly normal in appearance as it is shed from an anther. There is no abnormality visible in meiosis, where the usual 12 bivalent chromosomes may be observed in diakinesis. It is only when the pollen is used in the pollination of a pistil that the pollen-tube peculiarity becomes recognizable. Six or more hours after pollination one finds the stigma penetrated for a short distance by a large number of very short pollen tubes, which usually range between ½ and 2 mm in length. These remain short and do not function in fertilization, while the pollen tubes carrying the normal allelomorph of this gene have grown out long and continue growing through the style at the same rate as the pollen tubes of any normal 2n plant. Frequently the cell wall of the short tube is abnormally thick and transparent, the tubes are relatively broad, and usually do not burst. If the tests are conducted for 10–18 hours at 18–21°C the tips of the long normal pollen tubes are still to be found within the style, and if the pollen tubes in test slides are counted, a close approximation to a 1:1 ratio is found for these two kinds of gametophytes, long and short tubes. Figure 1 represents diagrammatically the appearance of a stigma with the short pollen tubes, and figure 2 shows a diagram of the pollen-tube distribution, which may be described as type V distribution (BUCHHOLZ and BLAKESLEE 1932a).

This gene will also manifest itself if the pollen taken from a *Datura stramonium* carrier is grown in the style of another species. An account of the behavior of pollen tubes in reciprocal pollinations between all possible pairs of ten species of Datura has been given elsewhere (BUCHHOLZ, WILLIAMS and BLAKESLEE 1935). The gene *s-1* may be recognized on the stigma and style of any species of Datura in which the pollen tubes of *D. stramonium* show a reasonable amount of growth. Even when the longer pollen tubes all burst, as is true in many of the interspecific pollinations such as *D. innoxia* pollinated with *D. stramonium*, the short pollen tubes with thick transparent cell walls and relatively broad diameter may usually be identified without difficulty.

Furthermore, we have pollinated *D. stramonium* plants carrying *s-1* with the pollen of *Datura quercifolia*, *D. ferox*, and *D. discolor*, and reared the hybrids. In half of these F_1 hybrids, one could easily recognize the presence of this *s-1* gene when tests of the pollen tubes of the hybrids were made, whether the tubes were grown in their own pistils or in the pistils of either parent. Even though a large proportion of the pollen of these crosses consists of shriveled pollen grains, the good pollen which is present segregates for *s-1* pollen tubes and other longer tubes. This gene, therefore, stubbornly retains its identity and its outstanding characteristics in a variety of interspecific hybrid combinations, as well as in crosses between genetic strains within the *stramonium* species.



FIGURES 1–5.—Diagrams showing pollen tubes in styles with stigmas at left. Also graphs of distribution of pollen-tube ends. Figure 1—Diagram of pollen tubes of carrier of gene *s-1*; Figure 2—graph of distribution of ends of pollen tubes of above; Figure 3—diagram of pollen tubes of carrier of *s-2*; Figure 4—diagram of pollen tubes of carrier of *sb-1*; Figure 5—graph of distribution of pollen tubes of above.

The record of its egg transmission during six generations is as follows: 358 non-carriers and 336 carriers among the 2n offspring of 2n carrier parents; 115 non-carriers and 119 carriers among the 2n offspring of 2n+1 carrier parents; 30 non-carriers and 45 carriers among the 2n+1 offspring of carriers. The ratios obtained were all disomic since the locus of the *s-1* gene was not borne by the extra chromosomes in any of the 2n+1 parents tested. These records include both selfs and female backcrosses and give a total of 503 non-carrier and 500 carrier offspring, which is the 1:1 ratio expected on the assumption that the gene does not adversely affect viability. A male backcross of a carrier to a normal female produced 47 offspring, none of which were carriers. This confirms the conclusion reached from the behavior of the carrier pollen tubes that the gene cannot be transmitted through the pollen. Because of this non-transmissibility through the male, selfs and female backcrosses give similar ratios.

Another gene very similar and almost as characteristic as the one described above is *s-2*, which also gives a type V pollen-tube distribution. It was obtained from a plant in the immediate progeny of pollen which had been treated with radium. The short pollen tubes of *s-2* are somewhat longer than those of *s-1*; they are more slender back of the end and somewhat irregularly club shaped, occasionally somewhat irregular or beaded near the end, as shown in figure 3. They do not have the conspicuous thick wall described for *s-1*. They are likely to burst after longer periods, but very few will burst within about 12 hours; not all of them will burst after prolonged periods of testing. We have found no external structural differences between the carrier plants and the non-carrier sibs. So far as its effects upon the external appearance of the plant is concerned, therefore, this gene acts like a recessive. It has been noted in many plantings, however, that the non-carriers tend to come into flower a few days earlier than the carriers, so that the effect of this gene on a sporophyte carrier may be physiological. When considered in this light, therefore, it may be looked upon as slightly dominant, since this physiological effect may be more or less detectable in a heterozygote.

The gene *s-2* may be present in a plant without giving the short pollen tubes in every test. Early flowers of such plants, when their pollen was tested, have been found to show half of the pollen ungerminated (type VII distribution); then a week later the same plants were found to give a high proportion of germinated pollen with the characteristic short tubes. However, the short tubes or ungerminated pollen never give normal growth or long pollen tubes under any conditions of testing. We must conclude that *s-2* is a gene which has more than one method of expression and test slides must be examined with care (and the tests giving type VII distribution repeated) lest a carrier be incorrectly diagnosed. The 50 per cent ungerminated pollen (type VII) is not to be considered a positive test for this gene. Causes other than genes may at times be responsible for poor pollen germination. If *s-2* is present in a plant, the pollen of another flower collected at a later time will usually give a positive diagnosis, unless the carriers suffer from drought or some other adverse environmental condition.

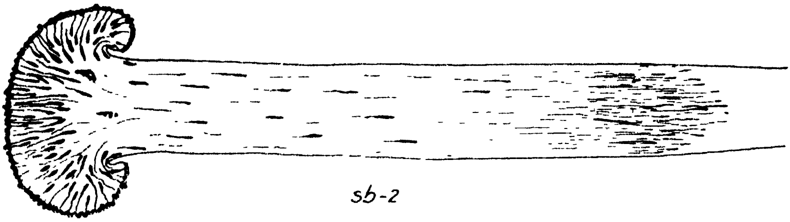
The gene *s-2* has also been recognized in the pollen of hybrids between *D. stramonium* and *D. ferox*, *D. quercifolia*, and *D. discolor*. If the tests are made at times when the pollen of a carrier gives satisfactory tests on styles of *D. stramonium*, the gene may be recognized when this pollen is grown in the pistils of other species.

The segregation records of *s-2* through egg transmission seem to vary. The first segregating planting from a self of the original parent gave 15 non-carriers and 17 *s-2* carriers. In the progeny of these carriers there were plantings in which the ratio was 62 non-carriers: 36 *s-2* carriers, and 32 non-carriers: 13 *s-2* carriers; the massed data (from 22 plantings with records on more than 6 plants up to 1935) gave 334 non-carriers: 228 *s-2* carriers among 2n plants. It is not unusual to have recessives run low in ratios due to their deleterious effect on viability and this would seem to be the case in the present instance. However, the ratios may have been affected by the method of sampling. We have found that the earlier flowering plants were more often non-carriers and those flowering a little later had higher proportions of carriers. It is possible that the unequal ratios of the above plantings may have been due in part to plantings made from incomplete seed germinations or to incomplete recordsof plantingswhere only a portion of the family was tested. In our latest stock planting (3401556), backcrossed 3 generations and 5 generations removed from the origininal plant, we had only 15 plants and 9 of them were *s-2* carriers, so that so far as this small number may indicate, there seems to be no shortage of *s-2* carriers. However, in all *s-2* plantings of 1935 combined, in which only a few plants in each planting were tested, the ratios recorded were 62 non-carriers: 41 carriers. Plantings with less than 6 plants determined were not included.

Another gene which we have recognized with ease in carrier plants is *sb-1*, which gives a type of pollen-tube distribution called VIa (BUCHHOLZ and BLAKESLEE 1932a) shown in figure 5. This gene gives short pollen tubes which burst within the region from 2–8 mm from the top of the stigma. The bursting is somewhat characteristic. The ends of the burst pollen tubes appear ragged or frayed, as if to suggest that the protoplasm is thin and flows up and down with ease along the surrounding elongated cells of the conducting tissue. Thus the burst ends have slivers or barbs extending forward and backward as shown in figure 4. The *sb-1* pollen tubes are almost never swollen or club-shaped, nor do they have a thick transparent wall as described for *s-1*.

We have attempted to recognize this gene when the pollen of a carrier is tested on the stigmas of other species. Grown in the styles of species which do not give bursting of the *D. stramonium* pollen tubes, it may be recognized, but on the pistils of *D. innoxia* and *D. metcloides*, where nearly all *D. stramonium* pollen tubes burst near the stigma, one could not be certain of recognizing this gene. Likewise, in making tests for carriers among interspecific hybrids it would be very difficult to recognize the gene *sb-1*, since there are usually many burst pollen tubes of all kinds from the pollen of such hybrids.

6.



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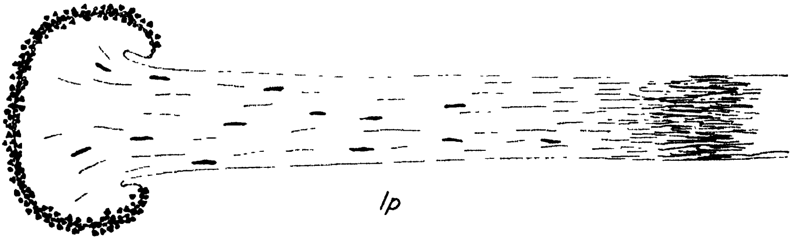

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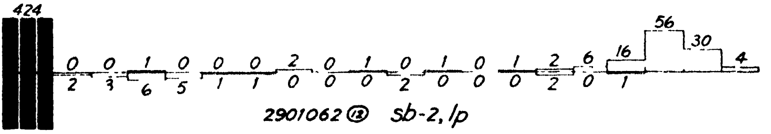


FIGURE 6.—Diagram of pollen tubes of carrier of gene *sb-2*.
FIGURE 7.—Diagram of pollen tubes of carrier of gene *lp*
FIGURE 8.—Graph of distribution of ends of pollen tubes of a carrier of both genes *sb-2* and *lp*.

Our segregation records of *sb-1* show a ratio of 1 non-carrier to 1 carrier in female backcrosses. For the 2n carriers pollinated with pollen of various genes and with the pollen of 2n+1 plants, the massed records of 7 progeny plantings have given 236 non-carriers: 234 carriers. The offspring of 2n+1 carriers which had been pollinated with pollen containing various genes or "prime types" have shown 115 non-carriers: 105 carriers in 11 plantings. The locus of the gene was not borne by the extra chromosome in any of these 2n+1 parents. The totals for these female backcrosses are 351 non-carriers: 339 carriers. The records do not include many small plantings, in which only a few plants were tested.

One of the more difficult to recognize, yet distinctive pollen-tube genes is *sb-2* (fig. 6). The pollen of carriers gives either a type VII or a type VIa distribution (BUCHHOLZ and BLAKESLEE 1932a). The bursting of pollen tubes carrying this gene is preceded or accompanied by a swollen or relatively large club-shaped end, and occurs in the region of the stigma. In fact, not all pollen tubes which carry the gene will burst; they may only stop growing and may remain abnormally swollen. The appearance of *sb-2* is very similar to the abnormal tubes obtained when the largest chromosome 1·2 is present as an extra (BUCHHOLZ and BLAKESLEE 1932). The difficulty offered by this gene comes from the fact that very often the pollen grains which carry *sb-2* fail to germinate, so that we may have the 50 per cent ungerminated pollen (type VII) as the characteristic of this gene for many weeks during the early part of the reproductive cycle of a plant. Plants beginning to flower in July usually do not show the swollen or burst pollen tubes for several weeks, giving type VII distributions instead of VIa, and in dry years it is not positively identifiable until past the middle of August. Very late in the season (September) the pollen of *sb-2* carriers may give the 50 per cent ungerminated condition again.

While we have had interspecific hybrids which were doubtless carriers for *sb-2* it would not be an easy task to identify this gene with certainty. As stated before, both ungerminated pollen grains and burst pollen tubes are to be expected normally in the pollen produced by a hybrid, and this gene could only affect the quantitative relations of these abnormal classes of gametophytes. Of course, a gene whose diagnosis is not easy within the species would be expected to offer difficulties in the hybrids with other species.

We should state that *sb-2* was first recorded as a gene giving 50 per cent ungerminated pollen. We happened to make a routine cross of an *sb-2* carrier plant with a white flowered type, planted the F_1's and selfed and backcrossed them to white. When seeds from some of these were planted, the linkage with *p* was apparent in selfs and male backcrosses. When pollen-tube growth in the carrier plants was studied late in August, the characteristic which would most certainly identify this gene was noted as a type VIa, or short burst pollen tubes, rather than the 50 per cent ungerminated pollen of type VII. The identity of this abnormality as supplying the pollen tube disturbances which give aberrant ratios in the segregation of *p* could be established beyond question.

The origin of *sb-2* was from the treatment of pollen with radium. The first of the carrier plants, plant number 2901062(12) was one in the immediate progeny of the treated pollen. The distribution of the pollen tubes of this plant is shown in figure 8, a type VII distribution, with an excess of ungerminated pollen. This was also a carrier for *lp*, a gene to be described below, and the fact that about three-fourths or more of the pollen was ungerminated suggested at once that this plant was heterozygous for two or more genes.

The segregation records in 12 plantings of selfs or female backcrosses in which six or more plants were tested have shown 100 non-carriers and 87 carriers.

Lobed pollen (*lp*) is a gene which occurred in plant number 2901062(12) that is, it was found in the same original plant which carried *sb-2*. Its characteristic feature is that the half of the pollen which remains ungerminated becomes swollen at the three germ pores of each pollen grain so that the ungerminated half of the pollen may be described as "lobed pollen" (fig. 7). We have called this a type VIIb distribution. This gene type was isolated for study because of the very unusual condition by which it may be recognized, and as one which gives a positive diagnosis for type VII which is characterized by 50 per cent ungerminated pollen grains. Sixteen plantings from carrier parents have given offspring with 169 non-carriers and 168 carriers.

This gene may be recognized in the pollen of hybrids made by pollinating a *D. stramonium* carrier with the pollen of another species. Approximately half of the hybrids will be carriers. The lobing is not always as pronounced, but it is an unmistakable condition, so that the *lp* type appears to have the same general stability as some of the other easily recognized gene types, *s-1* and *s-2*.

While *sb-2* and *lp* came originally from the same parent plant, these genes appear to be on different chromosomes. More recently we have obtained from the progeny of the same parent, a third unnamed gene, characterized by pollen tubes of the type VI which bursts within the upper half of the style. The new gene appears to have been carried along with, and partially masked by *lp* in many of our plantings.

METHODS USED IN LOCATING POLLEN-TUBE GENES IN PARTICULAR CHROMOSOMES

In attempting to discover which of the twelve pairs of chromosomes carry the different genes affecting pollen-tube growth, we found that we were more limited in our methods than with ordinary genes which are pollen-transmitted. These genes are egg-transmitted to half of the progeny only, so that tests to identify plants which carry the particular pollen-tube abnormality are necessary in each succeeding generation. A number of special methods for locating genes in particular chromosomes have been developed in connection with the Datura investigations (BLAKESLEE 1931). For the present problem four methods seemed available, although some of them could be used only in part.

Three of these methods are independent of a known gene in the chromosome tested and the other method depends upon the previous location of a gene in the chromosome. These methods are: (1) linkage with located genes; (2) linkage with such "prime types" as can be identified in the heterozygous condition by definite proportions of aborted pollen; (3) trisomic ratios, a method which is limited to the chromosomes which may be transmitted through the pollen as extras (11·12, 13·14, 15·16, 21·22, and 1·2 by way of 2·2); (4) hereditary behavior in transmission from a secondary trisomic to its primary. While we have made some use of all four of these methods, this last method was limited to the 1·2 chromosome.

Linkage with located genes

The method of linkage with known marker genes will be described first; and *sb-2* will furnish an illustration of how such linkages may be recognized.

TABLE 1

Offspring of parents heterozygous for pollen-tube gene (sb-2) and gene for white flowers (p).

♀ PARENTS	♀ BACKCROSSES TO WHITE (*p*)		♂ BACKCROSSES TO WHITE (*p*)		SELFS	
	PURPLE	WHITE	PURPLE	WHITE	PURPLE	WHITE
Non-carriers						
(Sb-2)$_2$ in pedigree 31091	36	43	92	90	240	87
Calculated	*39.5*	*39.5*	*91*	*91*	*245*	*82*
Carriers						
(Sb-2 sb-2) in pedigree 31091	239	215	185	700	287	242
Carriers in pedigree 3201679			188	642		
			373	**1342**		
Calculated for no linkage	*227*	*227*	*857.5*	*857.5*	*397*	*132*

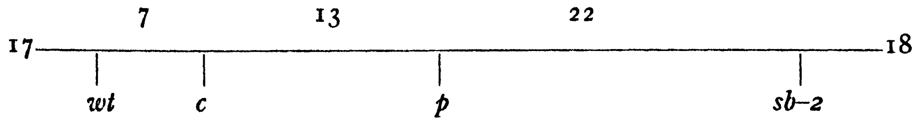
An *sb-2* carrier plant was pollinated with white (*p*). In the F_1 plants following this cross the *sb-2* carrier plants were identified. These were all heterozygous for white and were used in making male backcrosses to the recessive white. If the locus for this gene proved to be in the same chromosome as that for white (*p*) it would be in the chromosome carrying the dominant *P* and not in the chromosome carrying the allelomorph *p*. In male backcrosses, consequently, the *P* gametes, which also carry *sb-2*, would be eliminated during pollen-tube growth. The progeny would be all white except for the crossovers, which are purple. Table 1 shows the results for both male and female backcrosses and selfs.

There are actually a considerable number of purple plants or crossovers. Since only two classes appear, non-crossover whites and crossover purples, the percentage of crossing over is read directly. For *sb-2* and *p* it is close to

22 per cent. In practice we use the *sb-2* carriers in male backcrosses, which give the ratio of 373 purples to 1342 whites. If the non-carriers of *sb-2* are used in similar male backcrosses the ratio of purple to white is very nearly 1:1, our records for sib non-carriers being 92*P*:90*p*.

Linkage is seen also in the results from selfing which give 287 purples to 242 whites when 397 purples to 132 whites are the calculated values for a 3:1 ratio. The ratios from selfing indicate a lower crossing over value than the more accurate index from the male backcrosses. The non-carrier sibs when selfed have given 240 *P*:87 *p*, which is a proportion well within the probable error limits for a 3:1 ratio. When carriers were female backcrossed, the offspring showed a 1:1 ratio for purple and white without apparent effect of linkage. If the plants had been tested for pollen-tube growth, however, a higher proportion of purples than of whites would have been found to be carriers.

The *p* gene is in the 17·18 chromosome, as also the genes curled (*c*) and wilt (*wt*). It is also known that *c* and *wt* are in the ·17 half of the chromosome while *p* is in the ·18 half (BLAKESLEE 1929, 1930). The relative positions of these three genes on the 17·18 chromosome have been determined by their linkage relations (unpublished data, BLAKESLEE and AVERY). The gene *wt* in the ·17 half is about 7 units from *c*, and *p* in the ·18 half is about 13 units from *c*. To this linkage group we may now add the gene *sb-2* toward the end of the ·18 half. The data in table 1 indicate that *sb-2* is 22 units from *p*. Male backcrosses of *sb-2* carriers heterozygous for *c* gave 111 *C* to 205 *c* plants in the offspring, a proportion which indicates about 35 per cent crossing over between these two genes. A test with *wt* and *sb-2* showed only 33 per cent crossing over when somewhat more would have been expected, although there was a total of only 109 offspring in the backcross. The data as they stand are in general agreement with the arrangement of these four genes shown in the provisional map of the 17·18 chromosome given below.



The above method of linkages with various marker genes of known location was also used with the other pollen-tube genes. In many cases, carriers were pollinated with the pollen of plants homozygous for several marker genes in order to obtain F_1's heterozygous for both the pollen-tube gene and the markers. Male backcrosses were then made to the individual markers. Since no linkages were found for the other pollen-tube genes with the exception of the gene *s-2*, only this latter need be considered. Bronze (*Bz*), a dominant character, and ferox white (*fw*) are markers in the 3·4 chromosome. Male backcrosses of carriers heterozygous for these genes gave the following ratios: 30 *Bz*:35*bz* and 61 *Fw*:82*fw*. The gene for inermis (*a*) is in the 11·12 chromosome and the male backcrosses involving this gene gave the ratio 33 *A*:35 *a*. Male backcrosses involving curled (*c*), already discussed as in the 17·18 chromosome, gave the ratio 88 *C*:92 *c*. Male backcrosses involving pale-1 in the 21·22 chromosome gave the ratio 65 *Pl*:72 *pl*. Since the gene for tricarpel (*tc*), which is in the 15·16 chromosome, is transmitted poorly through the pollen, female backcrosses were used when this marker was involved and the *s-2* plants identified in the offspring by tests of pollen-tube growth. Among the *Tc* offspring the ratio was 31 *S-2*:21 *s-2* and among the *tc* offspring the ratio was 30 *S-2*:16 *s-2*. It will be remembered that the gene *s-2* runs low in female backcrosses. None of the above tests with gene markers showed any linkage. By this time, it had been determined by another method that the gene *s-2* is located in the 13·14 chromosome. Linkage tests were therefore made with albino-2 (*al-2*), which is a marker for this chromosome. For albinos the male backcrosses to the recessive are not practical without a supply of flowering albino branches grafted on normal stocks. In consequence, the linkage was calculated from segregation from self pollinations.

The pollen of a plant heterozygous for *al-2* was used on a heterozygous *s-2* female and in the progeny only the selfed seeds of *s-2* plants were saved and planted. The progenies of four of these *s-2* plants did not segregate for albino and were discarded, those of three of these gave albino segregation in the combined proportion of 260 normal: 134 albino, or 34 per cent albino. This ratio shows an excess of albinos. The calculated values for a 3:1 ratio are 295.5 *Al*:98.5 *al*, so that the excess of 9 albino plants per 100 is attributable to this linkage. The linkage value may be computed from the selfed progeny in this case with a fair degree of accuracy since there are only two classes of phenotypes, resulting from the two classes of gametes carried by the functional pollen tubes. The male non-crossover gametes all carry albino and only the crossover gametes carry the normal allelomorph of albino. If there were no linkage, the expected proportion from selfing would be 25 per cent albinos and if the linkage were close, let us say with less than one per cent crossing over, the expected proportion would be close to 50 per cent albinos. Thus the linkage scale (from 1–50 per cent crossovers) is read as the difference obtained between the limits of 50 per cent albino seedlings and 25 per cent albino seedlings. Since the increases in linkage values decrease the observed proportion of albino seedlings, the linkage scale reads in reverse order from the seedling scale.

Our observed albinos form 34 per cent, or 9 per cent in excess of the 25 per cent expected from selfing when linkage does not occur. This 9 per cent is nearer to the 25 per cent limit than to the 50 per cent limit on the scale and must be multiplied by 2 and subtracted from 50 in order to give the linkage value which would be expected in a male backcross. Our observed results, therefore, indicate a crossover value of 32 per cent between *s-2* and *al-2*, assuming that there are no other conditions present which would modify this ratio. The situation may perhaps be made clearer by giving the ratio of *Al* and *al* for the male and female gametes.

Female gametes—1 *Al*+1 *al*
Male gametes—32 *Al*+68 *al*
F_2 zygotes—32 $(Al)_2$+100 *Al al*+68 $(al)_2$ or 132 *Al*:68 *al*.

The ratio 132 *Al*:68 *al* equals 260 *Al*:135 *al*, which, except for a discrepancy of 1 due to disregarding decimals, are the numbers actually found in the backcross.

Linkage with prime types

The method of linkage with appropriate prime types has been used successfully for the location of the gene *s-2*, as also for the location of other genes in Datura (BLAKESLEE 1931). Prime types (BERGNER, SATINA and BLAKESLEE 1933), it will be remembered, are races in which certain chromosomes have been modified in terms of those of our standard Line 1. They include chromosomal changes brought about by segmental interchange and simple translocation, which give circles, chains and "neckties" of four or more attached chromosomes in the heterozygous condition. Most of the "neckties" are associated with 50 per cent aborted pollen grains some of the chains with 50 or 25 per cent aborted grains and a few of the circles with 25 per cent pollen abortion. The cause of the pollen abortion in some of these cases has been discussed (BERGNER, SATINA and BLAKESLEE 1933) but need not concern us here. The important fact for our present consideration is that plants heterozygous for such prime types can be identified by inspection of the pollen without resorting to cytological technique. Linkage of a gene with the modified chromosomes of such prime types can be used therefore to show that the locus of the gene in question is in one of its two or three modified chromosomes.

Our *s-2* carriers, which are in the standard Line 1, were used as females in crosses with the various prime type testers which show definite proportions of shriveled pollen grains in the heterozygous condition. Plants in the F_1 generation from this cross show the percentage of aborted pollen grains characteristic for the heterozygous prime type involved in the cross. An F_1 carrier was then identified and its pollen used in a male backcross to Line 1. Half of the male gametes will carry the Prime type chromosomes and half the unmodified Line 1 chromosomes. If no linkage is involved with *s-2* the Line 1 male gametes will unite with the Line 1 female gametes to produce plants homozygous for Line 1 and characterized by good pollen. Prime type male gametes, on the other hand, will unite with the Line 1 female gametes to produce plants heterozygous for the prime type and characterized by a definite proportion of aborted pollen. The results of the tests are shown in table 2, where linkages with the chromosomes involved in the several prime types are shown by + signs and lack of linkage is shown by − signs.

TABLE 2

Male backcrosses showing segregation of male gametes in s-2 carriers heterozygous for prime types.
A + sign indicates linkage with prime type tester, a − sign indicates no linkage.

PRIME TYPE USED	TYPE OF ♂ GAMETES		CHROMOSOMES TESTED BY BACKCROSSES											
	L 1	PT	1·2	3·4	5·6	7·8	9·10	11·12	13·14	15·16	17·18	19·20	21·22	23·24
3	37	40						−					−	
7	40	31					−					−		
11	4	79						+	+		+			
15	24	24		−						−				
16	42	44				−							−	
17	3	63					+		+					+
19	8	74		+					+					
30	3	106							+					+
34	74	75						−						−
36	25	73			+				+	+				

Prime type 3 has the chromosomes 11·12 and 21 22 modified. Prime type 7 has the chromosomes 9·10 and 19·20 modified. Backcrosses involving these two prime types give a 1:1 ratio of plants with good and with a characteristic percentage of aborted pollen and hence show that *s-2* is not linked with the modified chromosomes involved in these two prime types. The case is quite otherwise with prime type 11 which contains chromosomes modified from the 11·12, 13·14, and the 17·18 chromosomes. Out of the 83 plants from the backcross, 79 had 25 per cent aborted pollen and thus were shown to have come from prime type 11 male gametes while only 4 had good pollen and were thus known to have come from Line 1 male gametes. The gene *s-2* was originally in Line 1 and hence linked to Line 1 chromosomes. Unless the linkage is broken by crossing over, the Line 1 pollen of the F_1 plants will contain the chromosome bearing the *s-2* gene, so that their pollen tubes are kept back or sifted out when this pollen is used in a backcross. It is only the prime type 11 half of the pollen which remains functional and reaches the ovary. The progeny from this male backcross to Line 1, therefore, will be the result of the cross Line 1 by prime type 11 and all the plants will be recognizable as heterozygotes for prime type 11 by 25 per cent aborted pollen grains except for a few crossover plants which will have good pollen. As seen by the table, there were 4 or 4.8 per cent of such crossovers between *s-2* and prime type 11. It is thus shown that *s-2* is linked with prime type 11 and hence its locus is in one of the three chromosomes which have been modified in this prime type. In other words, its locus is in the 11·12, the 13·14, or the 17·18 chromosome. Linkage is shown again in tests with prime type 17 which enables us to say that *s-2* is located in the 9·10, the 13·14, or the 23·24 chromosome. It must therefore be in the 13·14 chromosome, which is the only one involved in both prime type 11 and prime type 17. As will be seen from the table, this conclusion is confirmed by tests involving four other prime types which contain modified 13·14 chromosomes. Prime types not involving the 13·14 chromosome do not show linkage with *s-2*. Negative evidence is not conclusive since the distance of the gene may be so far removed from the point which limits the linkage that 50 per cent crossing over may occur; and this cannot be distinguished from random assortment of unrelated chromosomes. All the chromosomes have been tested by the prime type method except the 1·2 chromosome and this chromosome has been tested by the trisomic method to be discussed later. In using the prime type method it is immaterial whether the F_1 is male backcrossed to Line 1, which was the usual procedure, or to the prime type. Either female parent would serve to identify the male gametes. In the first case the plants with good pollen would have come from Line 1 male gametes and those with definite proportions of aborted pollen from the prime type male gametes, while in the second case it would be the plants with good pollen that came from the prime type male gametes. In a few cases, such as prime type 34, the homozygous prime type is recognized by morphological peculiarities, a fact which enables one to record by inspection of the plants rather than by examination of the pollen when the prime type is used in backcrosses.

Tests by the prime type method are being made of the other pollen-tube genes that we have discussed in this paper. The results so far obtained appear to indicate that *s-1* is in the 9·10 or the 19·20 chromosome.

Method of trisomic ratios

From the beginning of our studies of the pollen-tube genes we have attempted to use trisomic ratios as a means of locating these genes. It may be recalled that for the gene tricarpel (*tc*) the trisomic method was used successfully (BUCHHOLZ and BLAKESLEE 1927), but in that case the 2n+1 plants could be rendered heterozygous for *tc* by direct transmission of this gene through the pollen of the homozygotes. Although *tc* suffers a high elimination in pollen transmission when it is obtained from a heterozygous plant and some even when it is homozygous, the F_1's may be obtained without great difficulty and the trisomic segregation may be observed in female backcrosses without the complications of the disturbance due to differential elimination of *tc* pollen tubes in male backcrosses.

However, the pollen-tube genes described in this paper cannot be transmitted through the pollen and their homozygotes are unknown. Our only practical method of combining $2n+1$ plants with the pollen-tube genes is to obtain carriers in crosses resulting from the successful pollen transmission of the extra chromosome.

We have indicated the possibilities of pollen transmission of extra chromosomes in previous papers (BUCHHOLZ and BLAKESLEE 1932, BLAKESLEE 1934). Special methods must be used, such as restricting the pollen and selecting the seeds from the lower half of the seed capsule. Where restricted pollinations were made, usually the lower half contained the largest proportion of trisomics. In some instances we mixed the pollen of three or four trisomic plants and used this pollen mixture sparingly in pollinations with the expectation that more than one of the $2n+1$ types used for the pollen would appear in the offspring. We have also transmitted extra chromosomes by a method of splicing styles (BUCHHOLZ, DOAK and BLAKESLEE 1932), but this method had not been perfected when we began the work reported in the present paper. From our efforts at pollen transmission with restricted pollinations we have been able to obtain $2n+1$ plants for the primary chromosomes 11·12, 13 14, 15 16, 21 22, and for the secondary chromosome 2·2. The latter may be used in obtaining its primary $2n+1\cdot 2$ as a carrier. Thus we should be able to test for trisomic segregation from five of the twelve chromosomes.

The segregation for *s-2* from three of the trisomics is shown in table 3. The numbers tested from the $2n+15\cdot 16$ were too small to be included and the 1·2 chromosome was handled by another method to be discussed shortly.

Had we relied on the trisomic methods alone, we would have failed to locate *sb-2* since this gene is on the 17·18 chromosome which is not pollen transmissible as an extra. Although we stated (BUCHHOLZ and BLAKESLEE 1932) that this extra chromosome gives indications of being pollen transmitted at times to a very limited extent, we have been unsuccessful thus far in several attempts to obtain $2n+17\cdot 18$ carriers of *s-1*, *s-2* and *sb-1*.

As our table 3 indicates, the segregation obtained from $2n+1$ carriers was 1:1 and did not differ essentially from the segregation of ordinary $2n$ plants except in the case of the $2n+13\cdot 14$ carrier. Here the expectation of a trisomic ratio of 2:1 among the $2n$ offspring and 1:2 among the $2n+1$ offspring is closely approximated. The evidence from trisomic ratios, therefore, confirms the evidence from linkage with prime types and from linkage with the visible character *al-2*. There seems little doubt that the gene *s-2* is in the 13·14 chromosome.

TABLE 3

Segregation from 2n+1 types heterozygous for s-2.

♀ CARRIERS USED IN CROSSES	OFFSPRING 2n NON-CARRIERS	OFFSPRING 2n CARRIERS	OFFSPRING 2n+1 NON-CARRIERS	OFFSPRING 2n+1 CARRIERS	NO. PLANTS NOT TESTED 2n	NO. PLANTS NOT TESTED 2n+1	NO. PLANTS GROWN
2n+21·22	36	29	3	1	8	7	85
2n+13 14	45	24	3	6	7	4	89
2n+11·12	26	21	12	6	1	1	67

We have found that the trisomic method is not a very practical one for the initial location of one of these pollen-tube genes. This method depends upon the distinction between a 2:1 and a 1:1 ratio and thus requires large plantings. Every plant moreover should be tested for abnormalities of pollen-tube growth, and this procedure entails a relatively large amount of labor in comparison with the other methods available.

Segregation from secondaries

A fourth method which was used is that of testing the inheritance of the pollen-tube gene from a secondary to its primary. The primary 1·2 chromosome is not pollen-transmissible, but the secondary 2·2 may be easily obtained by pollen transmission from restricted pollinations. An *s-2* carrier plant combined with a 2n+2 2 plant may be expected to give among the primaries which it throws some *s-2* 2n+1 2 plants. If all 2n +1·2 plants so obtained are *s-2* carriers without exception, it might indicate that the gene is on the 1 2 chromosome. Our tests by this method were all negative; there were both carriers and non-carriers of *s-2* among our 2n+1·2 plants coming from 2n+2·2 carriers. We could therefore conclude that, barring the possibility of crossing over on the ·2 half of this chromosome, the genes in question were not in 1·2. Since crossing over might occur between the extra 2·2 and a gene located on the ·2 half of the 1·2 chromosome, this method would be a certain test only for a gene located on the ·1 half of this chromosome, if the tests were repeated for at least ten to twelve different 2n+1·2 plants reared from 2n+2·2 carriers and these were found to be carriers without exception.

In the experiments which had for their purpose obtaining 2n+1 types heterozygous for the pollen-tube genes, some additional data on transmission of extra chromosomes through the pollen were secured. Thus, when a normal plant was crossed with a $2n+2\cdot 2$ male parent and a limited amount of pollen was used, a relatively high proportion of $2n+2\cdot 2$ plants (18.7 per cent) were secured in the offspring. The high transmission of the $2\cdot 2$ chromosome through the pollen is more striking when the progenies of seeds from the upper half of the capsule are considered separately. From the upper half of the capsule there was only one $2n+2\cdot 2$ plant from 41 seedlings while from the lower half there were 16 (or 32 per cent) $2n+2\cdot 2$ plants from 50 seedlings. The experience with this, as well as with the other $2n+1$ types, the extra chromosome of which can be carried through the pollen, shows that the pollen-transmission of the extra chromosome can be depended upon to yield a sufficiently high proportion of $2n+1$ plants to secure some trisomic carriers.

DISCUSSION

While these pollen-tube genes were all obtained in the progeny of irradiated plants, there is no reason to suppose that similar abnormalities might not arise spontaneously. If they should arise they would soon be lost in the gametophytic selection which purges the pollen in each generation. Pollen-tube genes would be transmitted through the eggs of a carrier to half of the progeny in each generation. In seven generations there would be less than 1 per cent carriers in the total progeny of a carrier plant. This fact, added to the probability that their spontaneous occurrence would probably be infrequent, would make the search for spontaneous mutations of this kind in inbred lines very discouraging from the start. We have not found genes of this kind in untreated materials, but we have not made a systematic search for them on a large scale. Our examination of hundreds of plants in various investigations has not led to the discovery of any of the pronounced and easily recognized genes of the category described here.

We have also examined the progeny of irradiated plants which, in spite of the treatment, gave normal pollen-tube growth, and we have found that such progenies had essentially normal pollen-tube growth in the subsequent generation. When pollen-tube genes of any type were first recognized in the progeny of treated plants they were usually found to segregate approximately in a 1:1 ratio through egg transmission, though the carrier plants were sometimes deficient. We have also observed one very pronounced pollen-tube abnormality which was not transmitted maternally.

As stated in our introduction, there is no connection between these pollen-tube abnormalities and the manner in which the irradiation was administered. The pollen-tube genes are not peculiar to pollen treatment. Abnormalities of various types have appeared in the progeny of irradiated seeds and of plants otherwise subjected to the action of radium and

X-rays. However, when treatment is applied to seeds and more mature plants in which there are already several cells in the meristematic stem tip, the various forks or branches of the plant which may develop subsequently often differ from each other. The flower bud examined cytologically is not necessarily the same as another one on the same plant used for a seed progeny. It is not certain that all parts of the same flower or seed capsule are genetically alike. It is only after one generation of seed progeny had been grown that the different meristematic growing points would be uniform so that one might advantageously begin in a series of analytical studies.

The peculiar advantage of pollen treatment as a source of these pollen-tube abnormalities, as well as other kinds of genes, lies in the fact that every branch of a plant in the immediate progeny, or all of its flowers, may be expected to be genetically similar. When pollen is treated, we may be sure that only a single treated cell, the sperm nucleus, contributes a haploid set of chromosomes and that there is a similar haploid set of untreated chromosomes contributed by the egg. Thus we not only save a generation in obtaining plants in which the cells are genetically uniform throughout, but we may observe the induced changes if any are present which show in pollen-tube growth, in the pollen of the immediate progeny of the cells treated.

When radium is used the treatment is very easily administered to the pollen, on account of the short distances of exposure to this agent; and with either the X-rays or radium the treatment may be given in a laboratory far removed from the plants which furnish or receive the pollen.

In spite of the very pronounced effect of the various genes described here on the pollen tubes which carry them they appear to have no effect on the segregation ratios of genes carried by other chromosomes. Of course, they operate to lessen the seed yield in the progeny, but this is partially compensated by the fact that pollinations are usually heavier than necessary to supply the pollen tubes needed in fertilization. It is only when they are found to be linked with genes of other types that they give rise to pronounced disturbances in genetic segregation ratios of the genes so linked. Since there are 12 pairs of chromosomes in Datura, the probability of linkage of any one of our pollen-tube genes with other genes would appear to be one in twelve. However, as is well known, linkage between genes with approximately 50 per cent crossing over would give the same results statistically as the independent assortment of non-linkage. The expectation of disturbances due to linkages of a random sample of other genes of unknown location with one of our pollen-tube genes would therefore be less than one in twelve. Our data on linkage studies have borne out this expectation.

EVIDENCE THAT POLLEN-TUBE ABNORMALITIES ARE DUE TO GENES

That the pollen-tube genes described in this paper are actually genes and not some other type of abnormality is indicated by several lines of evidence. (a) The pollen of these plants has no more shrivelled pollen grains than any other 2n plant. The pollen is normal in appearance as it is shed from the anthers. (b) The carrier plants have been examined by our group of cytological investigators (the first carriers as well as carriers four or more generations later) and have always been found to be normal at meiosis showing in diakinesis 12 bivalent chromosomes. (c) The genes follow a definite mode of inheritance, they are transmitted maternally only to half of the progeny. (d) They give segregation ratios which approximate the theoretical expectations which follow a gamete ratio of 1:1. The paternal segregation is recordable as the number of long normal and short abnormal pollen tubes, and the maternal segregation is recorded by the ratio of the non-carriers to the carriers, which is usually also a gametic 1:1 ratio. (e) They are like recognized genes for visible characters in that they may be located in definite chromosomes and show linkage phenomena with the accompanying crossing over. These pollen-tube genes, therefore, fulfill all of the usual tests for genes.

Some might suggest that these genes are deficiencies, deletions from chromosomes, or translocations. The fact that they are all recessives might also suggest deficiencies. If they are deficiencies the parts of the chromosome missing must be very small. Deficiencies involving only a half of a chromosome, or in some cases only the hump or satellite of a chromosome (BERGNER, SATINA and BLAKESLEE 1933) have been known to result in shrivelled pollen. However, the pollen grains which carry the pollen-tube genes appear to be perfectly developed. Furthermore, if the deficiency is large or if a translocation should be involved one might expect to observe this cytologically.

It should perhaps be mentioned that in certain cases in Datura (BLAKESLEE, AVERY and BERGNER 1935) excess chromosomal material has been found to behave in inheritance like a recessive and is distinguishable from a gene only by cytological examination. Recently, moreover, a pure-breeding type with extra chromosomal material has been synthesized (BLAKESLEE, BERGNER and AVERY 1936) which cannot be distinguished from the effects of a gene by the the usual cytological tests. We feel it desirable, therefore, to exercise a degree of caution in stating that we believe the pollen-tube abnormalities we have described are due to specific genes. The same caution, however, should be exercised regarding most other described genes.

SUMMARY

Five abnormalities in pollen-tube growth are described. Two (*s-1* and *s-2*) are characterized by very slow growth of tubes without bursting. Two (*sb-1* and *sb-2*) are characterized by slow growth with bursting. One (*lp*) has pollen grains which fail to germinate, but become lobed at the germ pores.

These abnormalities are not transmitted through the pollen but are transmitted through half the egg cells.

The pollen-tube type *sb-2* shows linkage with the genes *p*, *c*, and *wt* which have been previously shown to be in the 17·18 chromosome.

The type *s-2* has been located in the 13 14 chromosome (a) by linkage with certain prime types which show definite proportions of aborted pollen when heterozygous, (b) by trisomic ratios from $2n+13\cdot14$ plants which were heterozygous for *s-2*, and (c) by linkage with a gene (*al*) in the 13·14 chromosome.

The pollen-tube abnormalities are not associated with any recognizable chromosomal peculiarities, show linkage with known genes and are therefore believed to be due to genes.

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RECURRENT AUTO- AND EXOMUTATION OF PLASTIDS RESULTING IN TRICOLORED VARIEGATION OF HORDEUM VULGARE

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EXHAUSTIVE experiments conducted by Sô (1921) clarified the genetic nature of the white-striated 'Okina-Mugi', an agronomical variety of *Hordeum vulgare*, the results of which were referred to in one of my early publications (IMAI 1928). I have since verified his conclusions with hybridization experiments. In 1930, one of the variegated plants from a pure line showed a tricolored mosaic through additional coloration to yellow. Since the appearance of the tricolored stock I have extended my research in order to learn its nature. Last winter's cold severely affected and finally destroyed all the tricolored rosettes and some of their sister white-variegated ones, putting an end to the investigation to be here described.

The white-variegated barley behaves as a simple recessive, the variegation being linked very closely to the normal allele of contracted habit (Sô, OGURA and IMAI 1919). Although the gene itself is very constant, it stimulates the recurrence of plastid mutation from green to white, or in other words, exomutation of plastids occurs recurrently (IMAI 1936). The exomutation rate varies with environment (IMAI 1935). Since the white stripes or sectors are due to the propagation of mutant albinotic plastids, the white seeds give rise to albinos when they are germinated. In this plant the inheritance of plastids is maternal, the pollen tubes contributing no plastids to zygotes through fertilization.

In 1930, a white-variegated pedigree gave a seedling with green-variegated yellowish (tricolored) leaves along with 165 white-variegated (almost green at the seedling stage) sister seedlings. The mutant further grew to produce four variegated ears. The leaves of the adult plant are RIDGWAY's Light Greenish Yellow, variegated with Spinach Green stripes as well as with white ones, presenting a tricolored foliage. The yellow color varies with the condition of the leaves, the young leaves resembling Cosse Green, which fades away to Martius Yellow with age. Because of the fact that the yellow parts become very greenish, although the white stripes are very evident, usually the difference in the color of the ears is not very marked.

From the 1931 sowing, the seeds germinated with results as shown in table 1, in which will be found the data for progenies of the mutant and its sister white-variegated plants.

In this and subsequent tables the almost green (pseudogreen) seedlings which develop into white-variegated are classified as white-variegated while the green-variegated yellowish (bicolored) seedlings which later develop white stripes are classified as tricolored.

TABLE 1

MOTHER PLANT	NUMBER OF PLANTS	WHITE-VARIEGATED	TRICOLORED	YELLOW	ALBINOTIC	MOSAIC	TOTAL
White-variegated	123	25407	—	--	811	31	26249
Tricolored	1	150	17	35	3	0	205

Except for the occurrence of a few albinotic and mosaic seedlings, the white-variegated sister plants bred true to type. The tricolored mutant gave 150 white-variegated, 17 tricolored, 35 yellow, and 3 albinotic seedlings, that is, 73.2 per cent reversional individuals. The yellow seedlings die about three weeks after germination. The tricolored seedlings, the extent of variegation of which varies in all gradations, survive and grow further, except however those having small green parts.

Seeds of 32 white-variegated and 6 tricolored plants derived from the mutant pedigree were sown in 1932. The data collected from the seedling beds are shown in table 2.

TABLE 2

MOTHER PLANT	NUMBER OF PLANTS	EARS	WHITE-VARIEGATED	TRI-COLORED	YELLOW	ALBI-NOTIC	MOSAIC	TOTAL
White-variegated	32	268	12444	--	--	508	8	12960
Tricolored	6	white-varieg. 30	1351	—		57	0	1408
		tricolored 10	360	40	36	4	1	441

Six tricolored plants, which were mosaic for green, yellow, and white, shot out 40 ears. Of these, 30 ears were white-variegated and produced 1351 white-variegated offspring, together with 57 albinotic seedlings, whereas 10 ears were tricolored and gave 360 white-variegated, 40 tricolored, 36 yellow, 4 albinotic, and 1 mosaic. The tricolored plants thus failed again to breed true to type, producing many reversional white-variegated in their offspring. The green stripes or areas that occurred in the tricolored sporophytes are due to somatic mutation, the occurrence of which was so frequent that all the yellow ears were variegated, the offspring containing many mutated white-variegated seedlings. Somatic mutation occurs at every stage of plant ontogeny, the earlier mutation resulting in heavily green-variegated plants that bear many green ears with white stripes, and later mutation in slightly variegated plants. Some yellow seedlings may therefore be variegated and others without any apparent green stripes. Pure yellow seedlings, however, die before sending out second blades, without a chance to grow further by means of the mutated green tissues, which, if they had sufficiently developed at an early stage, would have made a full life cycle possible.

TABLE 3

	EARS	WHITE-VARIEGATED	TRICOLORED	YELLOW	ALBINOTIC	MOSAIC	TOTAL
Tricolored	4	171	38	37	0	0	246
Slightly tricolored	1	0	0	46	0	0	46

From white-variegated plants, 32 tests gave rise to 12444 white-variegated, 508 albinotic, and 8 mosaic seedlings. The recurrent mutation being of plastids, and not of genes, the reversional plants invariably bred true to white-variegated. The white-variegated shoots from tricolored plants behaved in the same way.

In 1933, the sowing was restricted to seeds collected from one tricolored plant, the others having been damaged by moths. The results obtained are shown in table 3.

The data confirm the mutable nature of the tricolor. The four ears that gave mixed progeny were variegated with green stripes, while the fifth ear was very slightly variegated, giving however all yellow seedlings that died at the seedling stage.

The full data were obtained in 1934 and 1935 by successive pedigree cultures, as shown in table 4.

TABLE 4

YEAR	MOTHER PLANT	NUMBER OF PLANTS		EARS	WHITE-VARIEGATED	TRICOLORED	YELLOW	ALBINOTIC	MOSAIC	TRICOLORED MOSAIC	YELLOW MOSAIC	TOTAL
1934	White-variegated	150		716	34249	—	—	1687	31	0	0	35967
	Tricolored	10	white-varieg.	8	423	—	—	14	0	0	0	437
			heavily tricol.	10	505	7	6	22	3	0	0	543
			tricolored	28	868	298	296	43	4	0	0	1509
			slightly tricol.	3	13	31	104	1	0	0	0	149
1935	White-variegated	30		162	3913	—	—	260	5	0	0	4178
	Tricolored	31	white-varieg.	84	2956	—	—	127	0	0	0	3083
			heavily tricol.	30	957	71	84	48	17	0	0	1177
			tricolored	74	1086	344	982	125	3	1	2	2543
			slightly tricol.	17	25	19	547	27	0	0	2	620

Tests made during these two years with offspring of 180 white-variegated derived from the tricolored mother plants, resulted in all producing the expected forms. The ears on 41 tricolored plants were classified according to the degree of variegation into 40 heavily tricolored, 102 tricolored, 20 slightly tricolored, beside 92 white-variegated. The last-named ears gave all pseudogreen (white-variegated) seedlings, except some albinos, while others segregated into yellow and its variegated forms.

Experiments and observations made during five years support the view of recurrent mutation of plastids as will be seen from the following results: First, crossing experiments conducted in 1935 revealed that tricolor variegation is transmitted only through the mother. The tricolored ears, when out-pollinated by self green, gave 32 white-variegated, 15 tricolored, 20 yellow, and 2 albinotic seedlings, while its reciprocal mating resulted in 82 normals. Except for the yellow and albinotic seedlings, they were transplanted to a field to facilitate further growth, but the severe winter killed these rosettes. Second, no ears were pure yellow, but variegated with green, indicating the recurrence of somatic mutation. If tricolor variegation was due to sorting out of mixed green and yellow plastids, pure yellow ears or tillers should have resulted, whereas scarcely any came under my observation, although a few leaves may have been yellow without green variegation. Third, too many tricolored seedlings were segregated to be regarded as the result of sorting out of mixed plastids. The green-and-white mosaic seedlings from white-variegated ears amounted to only 0.2 per cent, average (IMAI 1936), while the yellow-and-green (tricolored) seedlings were quite numerous (see tables).

TABLE 5

EARS	WHITE-VARIEGATED	TRICOLORED	YELLOW	TOTAL	ALBINOTIC
White-variegated	4730	—	—	4730	198
	100%	—	—	100%	—
Heavily tricolored	1465	95	90	1650	70
	88.8%	5·75%	5·45%	100%	—
Tricolored	2643	738	1388	4769	125
	55·4%	15·5%	29.1%	100%	—
Slightly tricolored	38	50	697	785	28
	4·8%	6.4%	88.8%	100%	—

The data on the offspring of the tricolored plants for five years have been summed up and shown in table 5. In the table, green-and-white mosaic, yellow-green-and-white mosaic, and yellow-and-white mosaic seedlings are included in white-variegated, tricolored, and yellow segregates, respectively. Somatic segregation is apparent from the data, while the extent of variegation in the mother ears is strongly correlated with the proportion of the white-variegated (pseudogreen) and yellow offspring.

From the foregoing descriptions it is clear that tricolored barley is conditioned by the following gene and plastids: The gene, which is recessive to normal, stimulates the recurrence of plastid mutation from green to white and from yellow to white. The plastids are exomutable from green to white and from yellow to white, and automutable from yellow to green. The occurrence of the original tricolored plant is due to sporadic plastid mutation from green to yellow. The mutant yellow plastids, owing to their automutable nature to become green in sporophytic ontogeny, resulted in a bicolor. Further, through stimulation of the gene carried by the stock, recurrent exomutation of plastids added to it white variegation. The scheme presented in figure 1 indicates the action and reaction of the gene and plastids.

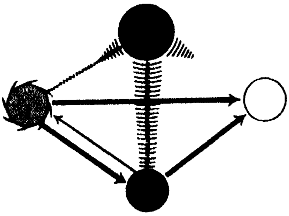

FIGURE 1.—The genetic mechanism of tricolored barley. The large circle is the nucleus and the small the plastids. The solid ripple-like lines indicate the action of the nuclear genotype in producing ample chlorophyll pigments in the plastids, while those extending only a short distance out from the centre of the nucleus represent the manner in which the plastid character manifests itself independent of the control of the normal nuclear genotype. The zigzag line joining nucleus and plastid indicates the effect of the former in changing the property of the latter The solid plastid is green, the dotted yellow, and the blank white. The vanes of the plastid show automutability. The thick arrows indicate recurrent mutation and the thin, sporadic mutation.

Plastid exomutation from green to white is almost restricted to the post-embryonic stage (IMAI 1928, 1935), while conditions differ when automutation occurs from yellow to green. The occurrence of green-and-white mosaic seedlings is believed to have started from egg-cells containing mixed plastids, and not from early mutation. The segregates, namely, the yellow-and-green seedlings, however, are too numerous to be attributed to the same origin, so that the majority of them should be characterized by green variegation as the result of early somatic plastid mutation. On this basis, plastid automutation from yellow to green occurs at all stages of somatogenesis, including embryonic ontogeny.

SUMMARY

Since white-variegated barley is caused by a recessive gene that stimulates the recurrence of plastid mutation from colored to white, exomuta-

tion of plastids results in white variegation. Through sporadic plastid mutation, green changed to yellow. The yellow plastids being automutable, frequently change to green, so that green variegation occurs in the yellow foliage. Since both green and yellow plastids are controlled by the stimulating gene, they transform into white plastids. Therefore, white variegation occurs in green and yellow tissues. The inheritance of yellow and white plastids is non-Mendelian, being transmitted maternally.

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ON THE GENETICS OF THE SPOTTED PATTERN OF THE GUINEA PIG

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INTRODUCTION

COAT patterns in which color is restricted to spots on a white ground are extremely common in guinea pigs, and have naturally attracted the attention of geneticists from the first. That the genetic situation is somewhat complicated is indicated by the fact that there are at present at least three decidedly different interpretations.

Variations in this type of pattern, ranging from mere traces of white in a few locations (toes, nose, between ears) to black eyed white, through intermediate grades showing much diversity in localization and often extreme asymmetry, were described by CASTLE (1905), who, however, attempted no genetic interpretation. GOODALE and MORGAN (1913) published data from which they concluded that "the spotted coat is a very complex affair depending presumably on a number of factors." They suggested the hypothesis that there may be one recessive factor (ss) necessary for any spotting with extension due to other factors (s_1s_1, s_2s_2, s_3s_3 etc.). IBSEN (1916) provisionally treated spotting as due to a recessive factor s, but noted "evidence is accumulating which seems to indicate that the relationship is not as simple as has hitherto been supposed."

In a list of guinea pig color factors (WRIGHT 1916) the symbol Σw was used for the "assemblage of unanalyzed factors which determine white spotting." In a review in the next year, however, (WRIGHT 1917), a pair of alleles S, s was listed as differentiating at least certain inbred stocks. The extreme variability of the pattern in both amount and localization of white even after 16 generations of brother-sister mating was cited as evidence for an extraordinary amount of non-genetic variability and as the reason for difficulty in genetic analysis. An effect of sex on spotting was noted. Females of each of 23 inbred strains were found to have slightly more white on the average than males. An effect of s as a modifier of the character of the tortoiseshell pattern of black and yellow was noted.

Early crosses between inbred strains

The data were not published in detail at the time. We shall present that bearing on the alleles S, s in condensed form. Twenty three strains were started in 1906 from single pairs by Mr. G. M. ROMMEL of the U. S.

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TABLE 1

Distributions of 4 inbred strains of guinea pigs, 1916–22 in percentages. Grade O means solid color: X means a trace of white. The grades are at 5% intervals (1 = 2.5 to 7.5%). W (black eyed white) is distinguished from 20 (a trace of color). The median percentages of white are given separately for males and females in the last two columns.

STRAIN	GRADE OF SPOTTING									NO.	MEDIAN % WHITE	
	0	X-2	3-5	6-8	9-11	12-14	15-17	18-20	W		♂	♀
34	100.0	0	0	0	0	0	0	0	0	333	0	0
39	0	35.2	32.9	14.3	7.7	6.7	2.3	0.9	0	659	13.9	24.4
35	0	0.8	4.0	10.1	19.7	29.4	27.1	8.6	0.3	1460	62.5	68.9
13	0	0	0	0.5	0.7	2.8	16.2	71.0	8.8	1278	95.0	96.4

Bureau of Animal Industry. These were maintained by exclusive brother-sister mating. The senior author took charge of the experiments in 1915 at which time 17 of the strains were still on hand. One of them (No. 34) had no white spotting (although much "silvering"). All of the others consisted wholly of tricolors but there were great differences among them in average amount of white. A drawing of the pattern of every animal was made at birth in a rubber stamp outline and the amount of white was estimated from an outline on tracing cloth divided into 20 squares. In table 1 is a condensed table of distributions in 4 strains. The distributions for 13, 35, 39, and two other strains have been published in full, separating males and females (WRIGHT 1926). Grade X means a trace of white and W, for black-eyed white, is distinguished from grade 20 with a trace of color. Strain 39 had least white of any of the spotted strains while 13 was at the opposite extreme.

During the years 1916 to 1919 many crosses were made between these and other strains (WRIGHT 1922). There were 1334 young from first crosses,

TABLE 2

The distributions of reciprocal crosses between strains 39 and 13, and of F_2. Note the similarity of F_2 to F_1 and the marked disagreement with expectation based on 25% strain 39, 50% F_1 and 25% strain 13 as indicated in the bottom line. In designating crosses in this and later tables, the female parent is given first.

CROSS	GRADE OF SPOTTING									NO.
	0	X-2	3-5	6-8	9-11	12-14	15-17	18-20	W	
39×13	0	3.3	0	13.3	6.7	30.0	20.0	26.7	0	30
13×39	0	0.8	6.4	8.0	14.4	25.6	27.2	17.6	0	125
Total F_1	**0**	**1.3**	**5.2**	**9.0**	**12.9**	**26.5**	**25.8**	**19.3**	**0**	**155**
F_2	0	2.4	12.2	13.4	8.5	23.2	24.4	15.9	0	82
Expected in F_2 if 1 factor	*0*	*9.5*	*10.8*	*8.2*	*8.6*	*15.6*	*17.5*	*27.6*	*2.2*	

981 from crosses of F_1 to a third strain, 692 F_2's from two strains and 617 F_2's combining 4 grandparental strains. Many more were born in selection experiments from these crosses. In all of these cases, spotted by spotted produced only spotted. We shall cite data from the cross between spotted strains at opposite extremes, (13×39) in the same condensed form as above (table 2).

Reciprocal F_1's are intermediate and F_2 is also intermediate and only slightly more variable than F_1. It is impossible to find in F_2 25 per cent segregants with the distribution of strain 39 or 25 per cent like strain 13. The *minimum* number of segregating factors may be estimated at 4 or 5. No upper limit to the possible number can be set. It may be added that while males and females showed the usual slight sex difference there were no indications of sex linkage.

Let us now turn to the crosses between the single self-colored strain (No. 34) and spotted strains, all with much white. The latter will be treated collectively (table 3).

TABLE 3

The distributions of reciprocal crosses between strain 34 and miscellaneous spotted strains, of F_2, and of backcrosses of self or near self segregants to the spotted strains

CROSS	GRADE OF SPOTTING									NO.		
	0	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W		0–5	6–W
34×spot	12.2	75.5	12.2	0	0	0	0	0	0	49	100	0
spot×34	11.1	77.8	11 1	0	0	0	0	0	0	18	100	0
Total F_1	**11.9**	**76.1**	**11.9**	**0**	**0**	**0**	**0**	**0**	**0**	**67**	**100**	**0**
F_2	9.4	53.1	9 4	12.5	0	6.2	9 4	0	0	32	71.9	28.1
1st Backcross	6.3	36.6	7.1	7.5	13.0	5.5	9.1	13.4	1.6	254	50.0	50.0
2–4 Backcross	8.6	31.9	6.9	6.9	7.7	11.2	9.5	16.4	0.9	116	47.4	52.6
Total Backcross	**7.0**	**35.1**	**7.0**	**7.3**	**11.4**	**7.3**	**9.2**	**14.3**	**1.4**	**370**	**49.1**	**50.9**

Complete dominance of self color is shown in only 12 per cent of the F_1 individuals but the average amount of white in F_1 is low, being always less than 27 per cent. In F_2, 28 per cent of the young exceed F_1, several being of high grade. There is a strong suggestion here of segregation of an incompletely recessive factor for high grade spotting. This is confirmed by the backcross of F_1 to inbred spotted. Exactly 50 per cent are above the limits of F_1. A second backcross of near self from the above matings to inbred spotted practically repeated the result from the first backcross. The same was true of small 3rd and 4th backcross generations. The young from these were derived only $\frac{1}{8}$ to 1/32 from the self strain but show no tendency to dilution of the heredity transmitted from the latter.

Non-genetic variability

The existence of the alleles *S*, *s* was first confidently asserted in a later paper (WRIGHT 1920) on the basis of a new experiment which had then reached the 7th backcross generation of self to inbred spotted. This experiment, ultimately carried to the 11th backcross generation, will be discussed later. The 1920 paper dealt primarily with an analysis of the variability within closely inbred strains. It was shown that in a typical strain (No. 35) descended at that time from a single mating in the 7th generation of brother-sister mating, there was variation from a trace of white to self-white but that this enormous variability (standard deviation about 22 per cent of the area of the coat) was almost wholly non-genetic. There was a correlation of only +.014 between parent and offspring (using a transformed scale designed to counteract the damping of variation in the neighborhoods of 0 and 100 per cent white). It was further shown that most of this non-genetic variability was of such a sort that environmental factors common to littermates played very little role. The correlation between littermates was only +.069.

While the intrastrain differences were thus proved to be non-genetic, the differences between strains are of course genetic. Both should be present in a random bred stock and this proved to be the case. The stock from which the inbred strains were derived had been maintained without even second cousin mating. In this stock the correlation between parent and offspring at this time was +.211 interpreted as indicating that 42 per cent (=2×.211) of the variance is genetic (leaving 58 per cent as non-genetic) on the assumption of no dominance or epistasis. Confirming this was the observation that the actual variance in the inbred stock was 57 per cent of that in the random bred stock on the transformed scale.

More extensive data on this question have been reported briefly in a later paper (WRIGHT 1926) together with data which indicate that the most important of the non-genetic factors common to littermates is the age of the mother. We shall discuss these and other data on the roles of heredity and environment in a later section.

There is a remarkable contrast between the situation in the guinea pig and that in the rat. In CASTLE'S (1916) selection experiments with hooded rats, the standard deviation among the offspring of the later generations averaged only about 3 per cent of the range from solid color to solid white. Yet progress by selection was possible up to the end, demonstrating the presence of considerable genetic variability. The correlation between mid parent and offspring (10th to 16th generations) averaged about +.25 and +.26 in the minus and plus series respectively. Thus a standard deviation of 3 per cent in an unfixed strain of rats is to be compared with one of about 20 per cent in a stock of guinea pigs in which all animals have been shown to have almost identical genetic constitutions. The same contrast is also doubtless indicated by the difference in regularity and symmetry. The patterns of hooded rats form a practically linear series of grades with a high degree of symmetry. In guinea pigs nearly every individual has its own characteristic pattern and there is usually asymmetry. Why the same character should behave so differently in different rodents is not known. The fact of an enormous amount of non-genetic variability in the guinea pig must be taken into account in any discussion of the genetics of white spotting in this animal. The phenotypes of single individuals are practically worthless here as indicators of genotypes.

Eleven generations of back crossing

The 11-generation backcross experiment, referred to above, was reported at the 1923 meeting of the American Society of Zoologists, but only a brief abstract was published. The first cross was between a self-colored male (unrelated to strain 34 or to the other inbred strains referred to above) and tricolor females from various inbred strains. Most of the young showed a little white spotting, the most extreme being a female with 30 per cent. None showed any yellow spotting. A small F_2 generation showed 25 per cent with spotting beyond the limits of F_1. F_1 was backcrossed with tricolors of various strains and produced about 50 per cent with no more white than F_1 and about 50 per cent with larger amounts. Each of these classes was about equally divided between animals with and without yellow spotting.

TABLE 4

Distribution of grades of spotting in F_1, F_2 and backcrosses to spotted strains, following a cross of a self-colored male to spotted females. The backcrosses are classified according to the amount of ancestry of strains 35 (median about 65% white) and strain 13 (median at 98% white).

	GRADES OF SPOTTING									NO.
	0	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W	
spot×self	26.7	70.0	0	3.3						30
F_2	10.0	45.0	10.0	10.0	10.0	5.0	10.0			20
Backcrosses to spot										
3/4 blood or more 35	7.7	44.3	7.7	17.3	7.7	1.9	9.6	3.8		52
Intermediate	1.8	33.9	8.8	5.8	10.8	11.0	14.3	12.8	0.8	398
3/4 blood or more 13	0	21.2	11.3	12.5	6.2	1.3	11.2	33.8	2.5	80

Selfs or near selfs of this generation were backcrossed again to inbred tricolors producing young which were 7/8 blood tricolor by ancestry but the distribution of types was substantially as in the first backcross generation. This process was repeated through 11 backcross generations, producing at length animals which were 99.98 per cent of tricolor ancestry but the results remained the same.

The tricolor strains used varied from ones which averaged about 65 per cent white (No. 35) to ones with over 95 per cent (No. 13). In table 4 all backcross generations are combined, but a distinction is made between those which were ¾ blood or more of strain 35, those which were ¾ blood or more of strain 13 and those which came of intermediate tricolor ancestry.

Obviously the line between *Ss* and *ss* must be drawn at different points, depending on the tricolor strain used. In table 5, the line was drawn above grades 3 and 4 for males and females respectively of the group with ¾ blood of strain 35, above 7 and 8 for the males and females respectively of the intermediate group, and above 11 and 12 for the males and females of those with ¾ blood of strain 13. These were the points which most nearly divided these groups on a 1:1 basis. It is of course recognized that this is somewhat arbitrary and that there was probably real overlapping in each case.

TABLE 5

Persistence of genes S and E through 11 generations of backcrossing of self (or near self) segregants ($SsEe^p$) to inbred tricolor stocks (sse^pe^p). Criterion for separating Ss and ss as described in text.

NO. OF BACK-CROSSES	AMOUNT OF SELF ANCESTRY	LITTLE OR NO WHITE		STRONGLY SPOTTED		TOTAL	
		NO YELLOW $SsEe^p$	TORTOISE Sse^pe^p	NO YELLOW $ssEe^p$	TRICOLOR sse^pe^p	Ss	ss
1	1/4	25	26	29	18	51	47
2	1/8	19	22	14	16	41	30
3	1/16	12	6	5	6	18	11
4	1/32	12	10	4	6	22	10
5	1/64	6	8	20	14	4	34
6	1/128	6	7	6	7	13	13
7	1/256	7	7	9	7	14	16
8	1/512	13	10	17	18	23	35
9	1/1024	15	14	18	9	29	27
10	1/2048	19	14	21	13	33	34
11	1/4096	5	4	4	2	9	6
Total		139	128	147	116	267	263

While no great stress can be put on the approximate equality of the groups with low and high grade spotting, the persistence of a bimodal distribution through 11 generations of backcrossing of selected low grades to pure tricolors can only be interpreted as evidence of transmission of an undilutable unit (*S*). The persistence of a group with no yellow spotting through the 11 backcrosses of course indicates transmission of a second unit (*E*) and the ratios indicate random assortment relative to *S,s*. The deficiency of tricolors is undoubtedly due to the failure of yellow spotting to appear in some of constitution sse^pe^p which were nearly or wholly white. The fact that the white spotting factor *s* behaves as a modifier of the tortoiseshell pattern was obvious in these data. In Sse^pe^p with little white, the yellow was usually restricted to scattered hairs or an irregular brindle. In sse^pe^p there was a more or less complete segregation of black and yellow into distinct spots on the white ground. These effects have also been noted by ILJIN (1928). The yellow spotting factor on the other hand had no apparent modifying effect on the white pattern.

Other interpretations of spotting

PICTET has also reported on extensive experiments with white spotting in guinea pigs. From his descriptions and illustrations, it seems clear that the patterns in his stocks are similar to those with which we have worked. His conclusions, however, are very different. He finds four major pairs of alleles, two affecting head only and two the trunk only, no one of which can be identified directly with our single major pair *S*, *s*.

In a paper (1925) dealing with white on the head he distinguishes self color, frontal white (self except for a median streak) and lateral white (white on cheeks as well as nose). Pure selfs by albinos gave 78 self to 82 frontal (approximately 1:1). Crosses between these F_1 types gave 54 self: 54 frontal: 36 lateral (exact 3:3:2) apart from albinos. He assumes two pairs of alleles with interactions as below.

	FF, *Ff*	*ff*
UU, *Up*	Frontal	Self
pp	Lateral	Lateral

It is assumed that the selfs were of type *UUff* and that all of the albinos happened to be *ppFf* (the albino factor being correctly interpreted as independent of spotting). Results from later generations are interpreted as in harmony with this hypothesis.

Later papers (1930, 1931) analyze spotting of the trunk. While it is stated that in most cases the extent of white is similar on head and trunk, it is held that these regions are subject to independent systems of genes because the correlation is not complete. In particular it is noted that animals with "generalized" white on the trunk may have a self colored head while one with a self colored trunk may have any amount of white on the head. Thus while the symbols *U* and *p* are used for genes affecting the trunk in these papers, they are apparently not considered to be related to the head factors assigned these same symbols in the earlier paper.

From the 1931 account, 5 original matings of self by spotted gave 42 spotted in F_1, and 52 spotted to 19 self in F_2, suggesting a dominant gene for spotting. On the other hand, 5 other matings of self by spotted gave 49 self in F_1, and 50 self to 19 spotted in F_2, suggestive of recessive spotting. The spotting in these two cases differed in character. The white in the recessive spotting was limited to the feet and small spots on the nape of the neck and in the sternal and perianal regions (apart from the head). This is called localized spotting. In the dominant type, the white was more or less extended over the body, ranging from narrow streaks across the back or along the belly to the completely white type (with black eyes). This is called generalized spotting.

The descendants of the above crosses were interbred. The total ratios agree with expectation from formulae assigned to parents according to the following scheme of combination effects.

	Pp, Pp	*pp*
UU, Uu	Generalized	Self
uu	Localized	Localized

($\chi^2 = 1.2$, $n = 12$ probability more than .999 of worse fit from random sampling.)

IBSEN (1932) makes the following statement in regard to white spotting. "White spotting (*s*) is quite variable in its expression in the guinea pig. There are probably a number of modifiers concerned and in addition there seems to be some variation that is entirely somatic. The dominant modifier *Fa* (face) causes the white spotting to be restricted entirely to the face, while its allelomorph, *fa*, permits white to appear in other parts of the body as well. Evidence has been accumulating which seems to indicate that there is a modifier of *Fa*, thus being a modifier of a modifier. This factor, *Na*, (narrow) causes the white face to be narrow while its allelomorph, *na*, permits it to widen. *Na* seems to be completely dominant to *na*. There are other types of white spotting in guinea pigs, such as white belt and white rump and it is even possible by selection to produce animals that are entirely white. None of these, however, can be readily fixed by selection thus lending support to the supposition that there is more than one pair of modifiers concerned in the production of each type. It would probably require much research to make a complete analysis of the modifiers affecting white spotting."

This scheme may be represented as follows. The data supporting it have not been published.

S — — —	Self
ss Fa — *Na* —	Narrow facial streak only
ss Fa — *na na*	Extensive facial white
ss fafa —	White on body as well as face

This scheme resembles that presented by WRIGHT in assigning one main factor to spotting but differs in treating this as completely recessive.

The greatest difference is with respect to non-genetic variability. IBSEN finds so little that definite formulae can be assigned certain phenotypes, while we have found so much in all inbred spotted stocks that such an assignment would be quite impossible.

New crosses between inbred strains

Thus since 1932 there have been three widely different interpretations of the genetics of white spotting of the guinea pig. It seemed desirable to make a new series of tests. The following experiments involve only three closely inbred strains.

Strain D has been closely inbred since 1906 first by Prof. W. E. CASTLE since 1916 by WRIGHT. It has never thrown white spotting of any sort.

Strain 2, as used here, is entirely descended from a single mating in the 15th generation of brother-sister mating (U. S. D. A strain, p. 759). It has consisted wholly of high grade spotted (median percentage of white 93.2 in males, 94.6 in females). There has been, however, considerable variability in grade as shown in table 6. The correlation of +.064±.025 between parent and offspring (here made without any transformation of scale, but arbitrarily treating black eyed white as a grade higher than grade 20 with a trace of color) shows that this variability is largely non-genetic (see table 16).

TABLE 6

Distributions of spotting in strains 2, 13, in reciprocal crosses between them and in F_2.

	GRADE OF SPOTTING									NO.	MEDIAN % WHITE	
	0	X-2	3-5	6-8	9-11	12-14	15-17	18-20	W		♂	♀
Strain 2				0.2	2.0	5.8	20.8	68.2	3.0	1650	93.2	94.6
Strain 13					0.1	1.2	12.5	62.4	23.8	1688	97.0	98.6
2×13							28.6	71.4		28	94.0	93.8
13×2						3.3	13.3	73.3	10.0	60	95.9	96.4
Total F_1						**2.3**	**18.2**	**72.7**	**6.8**	**88**	**95.2**	**95.4**
F_2					1.9		28.8	69.2		52	94.4	96.9

Strain 13 as used here traces to a single mating in the 18th generation of brother-sister mating. Two substrains are distinguished, 13 and 13E, the former but not the latter from continued brother-sister mating. There is, however, no recognizable difference between them in amount of white, which is the greatest of any inbred strain we have had. The median grades are 97.0 in males, and 98.6 in females. As with strain 2, the absence of appreciable correlations between parent and offspring (table 16) indicates that practically all variability is non-genetic.

Crosses have been made between strains 2 and 13 which indicate that

TABLE 7

Crosses involving only strain 2 (ss with median about 94 per cent white, table 6) with strain D (SS, always self colored).

MATING	AMOUNT OF D ANCESTRY	GENETIC CONSTITUTION	GRADE OF SPOTTING									NO	MEDIAN IN ss	
			0	X-2	3-5	6-8	9-11	12-14	15-17	18-20	W		♂	♀
2×D	1/2 D	*ss*×*SS*	91.5	8.5								59		
F_1×D	3/4 D	*Ss*×*SS*	95.7	4.3								207		
1 BC×D	7/8 D	(*Ss*, *SS*)×*SS*	99.0	1.0								305		
2 BC×D 3 BC×D	15/16 31/32 D	(*Ss*, *SS*)×*SS*	100.0	0.0								182		
F_1×F_1	1/2 D	*Ss*×*Ss*	46.2	23.1		3.8		23.1	3.8			26		
2 BC×2 BC	7/8 D	*SS*×*S*-	100.0									21		
		Ss×*Ss*	71.0	22.6	6.4							31	6.8	5.0
2 BC×7/8	7/8 D	*Ss*×*ss*	45.9	45.9	5.4		2.7					37		
7/8×7/8	7/8 D	*ss* ×*ss*	0	69.7	20.7	6.9	0.7	1.4	0.7			145	6.5	12.1
7/8×2	7/16 D	*ss* ×*ss*			14.1	18.7	26.6	20.3	12.5	7.8		64	47.5	55.8
(7/8×2)×7/8	21/32 D	*ss* ×*ss*		45.4	18.2	27.3	9.1					11	12.5	30.0

they are closely similar genetically. The distributions of F_1 and F_2 are shown in table 6. The medians (95.2 and 94.4 per cent for F_1 and F_2 males respectively and 95.4 and 96.9 per cent for F_1 and F_2 females respectively) give no indication of any complementary effect and the doubtful increase of variability in F_2 gives indication of only minor differences.

Strain 2 (*ss*) had been mated with strain D (*SS*) for another purpose and repeated backcrosses had been made to D (WRIGHT 1935a). F_1 (table 7) consisted of 59 animals (all *Ss*) of which 54 were completely self while 5 had a trace of white (one or both hind feet). This result from a cross to a strain as white as 2 indicates that D must have a rather exceptional stock of modifiers which repress white. Among 207 backcross animals (about half *Ss*, half *SS*) 198 showed no white while 9 had traces consisting in most cases of a few white hairs between the ears. Among 305 of the second generation of backcrossing to D (about 25 per cent *Ss*, 75 per cent *SS*) only 3 showed a few white hairs. 182 animals which were 15/16 or 31/32 blood of strain D were entirely self although it is probable that some were still heterozygous. Evidently *S* is almost invariably fully dominant over *s* where there is a preponderance of modifiers from strain D.

Some of the 7/8 blood animals (all self colored) were tested by mating with strain 13. Eight of these produced only self or low grade spotted among 83 young and were considered to be *SS*. The distribution of grades is shown in table 8. Two males and two females gave some high grade spotted, presumably *ss*, demonstrating the tested self parent to be *Ss*.

These tested heterozygotes were now mated *inter se* in order to extract a spotted line deriving its spotting from strain 2 but 7/8 of its modifiers from strain D (table 7). Their young consisted of 22 fully self and 9 low grade spotted. Matings of tested heterozygotes with these spotted segregants produced 17 fully self and 20 low grade spotted. Matings among these spotted animals and their descendants have produced 145 all spotted and, with few exceptions, of low grade. These results indicate complete recessiveness of spotting in animals which are 7/8 blood of strain D. Most of them were of what PICTET calls the localized type (where spotted at all on the trunk). The results are thus close to those which he has reported for his recessive spotting factor except that white on the head (few white hairs to extensive white on nose and cheeks) nearly always accompanied the localized spotting of the trunk, indicating that only a single main factor was involved.

These animals have necessarily derived their white spotting from strain 2 (with generalized white). The great difference in average grade of white must therefore be due to independent modifiers. Matings back to strain 2 have produced 64 young with median grade about half way between the parents.

TABLE 8

Crosses between strain 13 and animals which were 7/8 blood strain D, 1/8 blood strain 2.

	BLOOD OF 13	GENETIC CONSTITUTION	GRADE OF SPOTTING									NO.	MEDIAN % W			
			0	X-2	3-5	6-8	9-11	12-14	15-17	18-20	W		♂ *Ss*	♀ *Ss*	♂ *ss*	♀ *ss*
7/8 D×13	1/2	*SS*×*ss*	19.3	72.3	6.0	2.4						83	0.9	1.7		
		Ss×*ss*		46.2		7.7	7.7	7.7	23.1	7.7		13				
		ss×*ss*			20.0	20.0	40.0	20.0				5			42.5	72.5

TABLE 9

Repeated backcrossing of near self segregants to strain 13, illustrating persistence of gene S in spite of continued attempts at dilution. Reciprocal backcrosses are combined in this table

Ss×*ss*	BLOOD OF 13	GRADES OF *Ss* PARENTS	GRADE OF SPOTTING									NO.	MEDIAN % WHITE			
			0	X-2	3-5	6-8	9-11	12-14	15-17	18-20	W		♂ *Ss*	♀ *Ss*	♂ *ss*	♀ *ss*
1/2 (13)×13	3/4	All X	—	43.6	5.1	2.6	2.6	6.4	11.5	24.4	3.8	78	1.6	7.2	87.8	95.6
3/4 (13)×13	7/8	X-6	—	17.9	14.9	14.9	—	6.0	9.0	25.4	11.9	67	16.2	24.2	93.1	98.2
7/8 (13)×13	15/16	X-6	—	18.9	16.8	6.3	3.2	2.1	12.6	38.9	1.1	95	14.1	17.0	93.0	93.1
15/16(13)×13	31/32	3	—	16.7	25.0	8.3	—	—	8.3	25.0	16.7	12	16.7	31.2	96.2	96.7
31/32(13)×13	63/64	2-6	—	5.9	11.8	21.6	2.0	7.8	11.8	29.4	9.8	51				
63/64(13)×13	127/128	8-9	—	22.7	9.1	4.5	—	9.0	18.2	18.2	18.2	22				

TABLE 10

Tests of 15/16 blood segregants from backcross matings to strain 13 and of their inbred descendants. Low grades (Ss) produce a ratio which can be interpreted as 1 SS: 2 Ss: 1 ss while high grades (ss) breed almost like strain 13.

SOURCE	MOTHER CONSTITUTION AND GRADE	FATHER CONSTITUTION AND GRADE	GRADE OF SPOTTING									NO.	MEDIAN % WHITE			
			0	X-2	3-5	6-8	9-11	12-14	15-17	18-20	W		♂ Ss	♀ Ss	♂ ss	♀ ss
15/16 backcross	*ss* (16-20)	*ss* (16-20)					0.8	2.3	29.0	56.5	11.5	131			92.5	96.5
Descendants	*ss* (14-20)	*ss* (17-19)						8.2	20.4	53.1	18.4	49			93.9	95.0
15/16 backcross	*Ss* (1-9)	*Ss* (X-8)	12.5	31.6	11.8	10.3	2.2	2.9	11.0	13.2	4.4	136	12.0	28.5	88.4	97.7
Descendants	*Ss* (1)	*Ss* (X)	22.2	50.0	11.1			5.6		5.6	5.6	18				
	S- (0-1)	*SS* (0-X)	26.3	69.7	3.0		1.0					99				
	SS (0-1)	*ss* (15-20)		50.0	30.0	10.0	10.0					10				
15/16 backcross	*ss* (16)	*Ss* (9)			20.0			20.0		60.0		5				
Descendants	*ss* (19)	*Ss* (14)		30.0				10.0	40.0	20.0		10				

We will now turn to the descendants of the test crosses to strain 13 (table 9). Animals with only a trace of white were selected and backcrossed to 13 (*ss*). The process was repeated for several generations. No fully self-colored animals have appeared in these backcrosses. A markedly bimodal distribution of grades of spotting has, however, persisted throughout. In those 7/8 blood or more of strain 13, the median of the lower group (*Ss*) was, however, higher than that of the extracted spotted (*ss*) with 7/8 blood of strain D, while the median of the higher group is nearly the same as that of pure 13. As in the previously reported experiments of this type, there can be little doubt of the segregation of a single major unit factor. As a critical test, however, matings were made within each of the groups of supposed segregants (table 10). The matings between high grade spotted (grades 16 to 20) gave an array almost like that of pure 13. These were clearly *ss*×*ss*. Supposed heterozygous segregants (grades X to 9) of these same generations were also mated *inter se*. The progeny were utterly different. Of the 136 young, 17 were fully self and 24 more had only a trace of white. About 66 per cent had more color in the coat than the most colored of the young from the high grade parents. This result leaves no doubt of the conclusion that there is here segregation of a single spotting factor showing incomplete dominance over self. The results from the crosses between 2 and 13 show that it is the same spotting factor that was completely recessive where there was 7/8 blood of strain D.

As noted 17 of the 136 young from *Ss*×*Ss* were recorded as self. This is only 12.5 per cent or half the proportion of *SS* expected, indicating that a small amount of white may be brought out by the "modifiers" of strain 13 even in the absence of the main spotting factor. Unfortunately strain 13 is silvered as well as spotted. The above 17 animals showed more or less silvering but no white in the regions (nose, forehead and feet) where piebald white is most likely to appear. Those recorded as having a trace or more of piebald white all showed white in one or more of these regions.

Tests of grading

It seemed desirable to test the reliability of the grades. As noted, these were based on drawings made in rubber stamp outline. Most of the drawings were made by the senior author at the time of recording births. The estimates of grades were made by the junior author, using an outline on tracing cloth, divided into squares. In 306 cases (all from backcrosses of *Ss* to *ss* of strain 13 and including all grades from a trace of white (x) to black eyed white (w) estimates were made later by the senior author without knowledge of Mr. CHASE's grades. This comparison tests the accuracy of grading but not, of course, of the drawings. The statistical analysis of the two series of grades (not using Shepard's correction) gave the following results.

	Mean	*Standard Deviation*	*Correlation*
Chase	11.41	7.96	.9978 ± .0003
Wright	11.36	7.93	

There is no indication of any differential systematic errors and random errors are of negligible importance. Only about 0.2 per cent of the variance is determined by random errors.

In another test, Mr. CHASE made wholly independent records of the patterns of 119 miscellaneous animals, distributed rather uniformly from grade x to 20, but including no black eyed whites. The two sets of drawings were also graded independently.

	Mean	*Standard Deviation*	*Correlation*
Chase	9.99	6.53	989 ± .002
Wright	10.11	6.52	

There can be no important differential systematic errors either in drawing or grading. Random errors are naturally considerably increased but yet account for only one per cent of the variance. As the standard deviation in this population was nearly twice that of an isogenic stock with mean near grade 10, it may be estimated that about 3 or 4 per cent of the non-genetic variance may be attributed to the combined errors of drawing and grading.

Aberrant results

Results which at first seemed rather aberrant have been obtained from crosses between two stocks which are not related to any of the inbred strains of the preceding experiments. Strain A was built up to be dominant in a large number of genes. It consists typically of self agoutis (*SEACPFB*) with smooth fur except on hind toes (*RM*). Strain B was made up to be recessive in most of these respects. It consists of smooth-furred pink-eyed, dilute brown-yellow-white tricolors (se^vac^kpFbrm). The results of a large backcross generation have been described in a previous paper (1928). We are indebted to Dr. STRANDSKOV for use of recent data from these stocks and their crosses.

The earlier records of strain A naturally revealed heterozygosis in many respects including *S,s*. Since 1926, however, only low grade spotting has appeared. The complete records, 1926–1934 inclusive, are given in condensed form in table 11. The records for strain B are given for the years 1924–25 as reported previously and separately for the period 1926–34. The previous paper reported F_1 (B×A) only from 3 males of strain A, demonstrated to be homozygous *SS*. These records, it turns out, give a far from adequate picture of the situation. The total records for F_1 (B×A) and (A×B) 1926–1934, are reported here. The backcross data (F_1×B) are merely repeated (in condensed form) from the previous paper.

The first question raised by these data is whether *ss* can ever be completely self-colored. No such case was present in the data described earlier in this paper although the median grade of white was very low in certain strains (39,7/8D) and occasional animals had only a few white hairs. The records of 851 animals from strain B from 1926 to 1934 agree in this respect, but in the earlier records of B, 4 animals out of 454 (about 1 per cent) were recorded as completely self colored although from homozygous spotted parents. Unfortunately this question was not definitely in mind at the time of record and there may have been less care in looking for traces of white than in later experiments. We think it probable that plus modifiers may occasionally bring about complete absence of white spotting in *ss* but hesitate to affirm this positively until a case has been examined with this point in mind and has been tested genetically.

TABLE 11

White spotting in strains A, B and crosses All A's are believed to be SS in spite of some low grade spotting (up to grade 2), all B's ss in spite of 4 selfs (grade 0) Note segregation of self in F_2 from strongly spotted F_1's

SOURCE	REMARKS	GRADE OF SPOTTING									NO.
		0	X–2	3–5	6–8	9–11	12–14	15–17	18–20	W	
A	20 Matings (0×0)	100.0									96
	17 Matings (0×0)	86.2	13.8								217
	7 Matings (X×0)	73.2	26.8								41
	Total	**88.4**	**11.6**								**354**
B	1924–25	0.9	5.7	9.0	7.7	13.2	20.7	18.3	24.4	—	454
	1926–34	0	5.9	9.4	10.9	15.9	20.0	17.0	20.7	0.2	851
F_1	A×B low	36.9	63.1								160
	A×B high		87.5	12.5							16
	B×A low	29.8	70.2								242
	B×A high	16.7	69.8	5.2	3.1	2.9	1.2	1.0	0.2		420
	Total	**24.0**	**69.0**	**2.9**	**1.6**	**1.4**	**0.6**	**0.5**	**0.1**		838
F_1×B		9.8	37.8	12.4	8.9	6.6	9.4	8.7	6.4	0.0	437
F_2	From selected F_1's (grades 9–12)	17.2	41.4	6.9	3.4	10.3	6.9	3.4	10.3	0.0	29

Other questions are raised by the traces of white in strain A itself and the very high grade white spotting (up to 95 per cent white) found in some of F_1 (B×A). In strain A only self-colored males have been used since 1926, but in 7 cases females were used which had traces of white. Six of these females produced at least one offspring each, with traces of white. The other had only 2 young. The total ratio was 30 self to 11 with traces of white. The remaining matings of strain A (all self by self) may be divided into two groups: 20 matings with 96 young all self-colored, and 17 matings with at least 1 white-marked offspring each and a total ratio of 187 self to 30 with white. Two of the latter had white belts across the shoulders (grade 2).

If white spotting were due to a simple recessive in this stock, we should expect more than 50 per cent from matings classified as *Ss*×*ss* by the occurrence of at least one spotted young one, but only 27 per cent were observed. We should expect more than 25 percent from matings similarly classified as *Ss*×*Ss*, but only 14 per cent were observed. This hypothesis is ruled out unless *ss* is frequently self-colored, which is contrary to all previous experience. The traces of white in strain A are clearly not due to *ss*.

The next possibility is that these white-marked animals are *Ss*. If this is true, many selfs in the strain must also be *Ss*, so many that a considerable number of reasonably high grade spotted (*ss*) should have appeared. But this has not been the case since 1926. More evidence on this hypothesis is provided by the crosses with strain B. Table 11 distinguishes matings which produced at least one offspring above grade 2 (12.5 per cent white) from those which did not and in which the parent from strain A was certainly *SS*. In the former there may be a suspicion that it was *Ss*. However, the young above grade 2 from these matings constitute only 13.5 per cent of the total, a proportion so low as to practically rule out this hypothesis. It may be noted that the low ratio applies not only to the total but to every individual mating. The most extreme spotted (over 50 per cent white) came from 9 males of strain A. These produced the following ratios (making the cleavage at 12.5 per cent): 20:10, 28:7, 22:4, 31:3, 16:1, 25:3, 25:5, 6:1 and 2:1. None of these is in harmony with the mating formula *Ss*×*ss*. Thus it is strongly indicated that all of strain A, self and white-marked alike, were *SS* and that these strongly marked F_1's from (B×A) reaching 95 per cent white in one case, as well as the selfs (24 per cent of the total) were all *Ss*.[1]

This is confirmed by a number of tests. Matings between rather strongly marked F_1's (grades 9 to 12) produced the F_2 population in the bottom line of table 11. The appearance of as many as 5 self and 12 with only a trace of white out of 29 young make it certain that the F_1 parents were *Ss* (not *ss*). In other cases F_1's of various grades were mated with strain 13 (the whitest of the inbred strains) (table 12). There is no appreciable difference in the results from low grade and high grade F_1's and both agree with expectation from *Ss*×*ss* and not at all with that from *ss*×*ss*.

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[1] These crosses were in part from an experiment in which Dr. STRANDSKOV was testing the effect of X-radiation of males and many of the highest grade F_1's were from treated males. Fourteen males produced 12 above grade 2 out of 163 young before raying and 29 above grade 2 out of 191 after raying, a difference with a probability of being exceeded by random sampling of only .02. There is some suggestion here of a modifying effect.

TABLE 12

Tests of F_1 (A×B) of widely different grades by mating to the most extreme strain No. 13. Both groups of F_1's breed like Ss.

	GRADE OF SPOTTING									NO.
	0	X 2	3-5	6-8	9 11	12-14	15 17	18-20	W	
F_1 (2-6)×strain 13	0	32 0	10.0	6.0	4.0	8.0	16.0	24.0	—	50
F_1 (13-17)×strain 13	0	20 4	12.2	4 1	10.2	10 2	14.3	28.6	—	49

These results from (B×A) may be compared with those in table 13 from the mating (B×D). It will be recalled that strain D was wholly self (*SS*) and gave results in crosses with the high grade spotted of strain 2 which indicated an expectional array of modifiers which reduce white.

TABLE 13

Crosses of strains B and D.

		GRADE OF SPOTTING										NO.
		0	X	1-2	3-5	6-8	9-11	12-14	15-17	18-20	W	
F_1(B×D)	*ss*×*SS*	77 9	22.1									68
F_2(B×D)	*Ss*×*Ss*	57 3	24 2	4 4	5 2	3.9	2.3	1 0	1.3	0.3		384

The results from (B×D) are quite as expected. The white-suppressing modifiers of strain D have reduced *Ss* to a distribution similar to that of *SS* of strain A, and the *ss* segregants in F_2 must have a distribution much like that of *Ss* of F_1 (B×A).

Analysis of variability

Having established that there is just one major pair of alleles affecting white spotting in a number of diverse stocks, it is desirable to return to the evaluation of the roles of heredity and environment in determining the variation within a spotted stock. In doing this it is desirable to allow for the obvious damping of variability at both extremes of the range from self color to self white. An approximate method of doing this has been discussed in previous papers (WRIGHT 1920, 1926). The assumption that the pigmentation tendencies of the various areas of the coat are distributed normally leads to the transformation $X' = \text{prf}^{-1}(X - .50)$ where X is the proportion of color in the coat and prf^{-1} is the inverse probability function.

For the purpose of calculating the correlations, the grades were grouped in pairs and a transformed grade was found for the mid point of each (table 14). Table 15 shows the correlations between parents, between parent-offspring by sex and between littermates also by sex in a random bred stock and in a portion of strain 35 derived from a single mating in the 12th generation of brother-sister mating, using the above transformation of scale. The records for these two stocks were made during the same period of years (1916 to 1924) and are thus strictly comparable. The same set of correlations but without the transformation of scale has been found for a more recent branch (1926–1934) of strain 35, tracing to a single mating in the 22nd generation (35 D) (table 15) and for strain 2 and the two branches of strain 13 described earlier in this paper (table 16). In the case of 35 D, the correlations among brothers and sisters which were not littermates were also found. The correlations between brothers and between sisters were calculated from symmetrical tables, each pair being entered twice.

TABLE 14

Transformed grades, based on the inverse probability function of the midgrades and used in calculating standard deviations and correlation coefficients.

GRADE	MIDGRADE PERCENTAGE (X)	TRANSFORMED GRADE X′ $\left(X' = \text{prf}^{-1}\frac{(X-50)}{100}\right)$
X– 2	6.25	−1.534
3– 4	17.5	− .935
5– 6	27.5	− .598
7– 8	37.5	− .319
9–10	47.5	− .063
11–12	57.5	+ .189
13–14	67.5	+ .454
15–16	77.5	+ .755
17–18	87.5	+1.150
19–20	96.25	+1.780

In no case was there a significant amount of assortative mating. There are clearly significant correlations between parent and offspring in the random bred stock (average +.191) but none that are significant among the inbred strains (grand average +.016). The littermate correlations (+.282) are larger than the parent-offspring correlations (+.191) in the random bred stock; and though small are clearly significant among the inbred strains (grand average +.079). These results can only be due to environmental factors common to littermates. This interpretation is confirmed by the absence of positive correlation (−.048) between siblings which were not littermates in the inbred strain 35 D.

For analysis of the variability we can use only the data based on the transformed scale. If h^2 represents the portion of the variance due to heredity, e^2 that due to environment common to littermates and d^2 that due to environment not common to littermates; the correlation between parent and offspring should equal $\frac{1}{2}h^2$ if the effects of all genes combine additively (no dominance or epistasis) but should be somewhat less if these conditions are not met (WRIGHT 1920). Under the same conditions the correlation between littermates would be $\frac{1}{2}h^2+e^2$. The assumption of complete dominance and equal frequencies of alleles reduces the parent-offspring correlation to $1/3h^2$ and that between littermates to $5/12h^2+e^2$. In all cases, dominance reduces the genetic component of the fraternal correlation just half as much as it does the parent-offspring correlation (PEARSON 1909; WEINBERG 1910; FISHER 1918). Any non-additive effects among genes which are not alleles (epistasis) also reduces the correlations between relatives (WRIGHT 1935).

TABLE 15

Parent-offspring and fraternal correlations in a random bred stock, and in the inbred strain No. 35 at two periods. Transformed grades used in case of random breds and the earlier data from strain 35. Untransformed grades used in 35 D.

		RANDOM BRED STOCK		STRAIN 35 (1916–24)		STRAIN 35 D (1926–34)	
		NO	*r*	NO.	*r*	NO.	*r*
Father-Mother		143	+.060	140	+.064	72	−.047
Parent-offspring	♂-♂	973	+.244	738	+.015	200	−.068
	♂-♀	929	+.187	688	+.074	190	+.149
	♀-♂	1014	+.217	738	+.013	196	+.052
	♀-♀	965	+.116	688	−.004	185	−.031
	Average	**3881**	**+.191**	**2852**	**+.024**	**771**	**+.026**
Littermates	♂-♂	537	+.355	340	+.128	91	+.024
	♂-♀	1050	+.288	722	+.089	180	+.109
	♀-♀	493	+.190	305	+.107	89	+.218
	Average	**2080**	**+.282**	**1367**	**+.103**	**360**	**+.115**
Sibs not littermates	♂-♂					335	−.061
	♂-♀					509	−.049
	♀-♀					202	−.021
	Average					**1046**	**−.048**

The average standard deviation of males and females in the random bred stock was .757, that in strain 35 was .583 (transformed scale). The variance of strain 35 is thus 59 per cent $\left(\frac{.583^2}{.757^2}\right)$ of that of the random bred stock indicating that about 41 per cent of the latter variance was due to heredity, and had been lost after long continued inbreeding of strain 35. The correlation between parent and offspring in the random-bred stock (+.191) indicates 38 per cent (=2×.19) of the variance as due to additive gene effects. This differs so little from the 41 per cent estimated from the actual variances that little complication from non-additive gene effects (dominance or epistasis) is indicated. It should be said, however, that another method of calculating the transformed standard deviations of the inbred and random bred strains was made by fitting the distributions by the function $\text{prf}^{-1}(\Sigma f - .50) = 1/s\,[\text{prf}^{-1}(X - .50) - a]$ where a and s are the mean and standard deviation on the transformed scale and Σf the running sum of the fractional frequencies (WRIGHT 1926). This yields 54 instead of 59 per cent as the ratio of the variances (s=.782 in random bred stock, s=.574 in strain 35). A somewhat greater role of non-additive gene effects is indicated by this method. The difference was due mainly to irregularities in the distribution of the random bred animals.

TABLE 16

Parent-offspring and fraternal correlations in 3 inbred strains, characterized by very large amounts of white. Untransformed grades.

	STRAIN 2 (1926–34)		STRAIN 13 (1926–34)		STRAIN 13 E (1926–34)		TOTAL	
	NO.	*r*	NO.	*r*	NO.	*r*	NO.	*r*
Father-Mother	94	+.019	86	+.220	105	+.027	285	+.089
Parent- ♂-♂	402	+.035	486	+.007	368	−.078	1256	−.012
offspring ♂-♀	437	+.149	436	+.036	375	−.132	1248	+.018
♀-♂	408	+.044	488	+.041	366	−.019	1262	+.022
♀-♀	433	+.029	436	−.049	375	+ 029	1244	+.003
Average	**1680**	**+.064**	**1846**	**+.009**	**1484**	**−.050**	**5010**	**+.008**
Litter- ♂-♂	179	+.098	250	+.096	188	+.011	617	+.071
mates ♂-♀	372	+.074	451	+.090	358	+.005	1181	+.059
♀-♀	215	+.068	213	+.025	166	+.054	594	+.049
Average	**766**	**+.078**	**914**	**+.077**	**712**	**+.018**	**2392**	**+.060**

The females exceeded the males in amount of white by .241 on the transformed scale in the control stock. This is 31.3 per cent of the standard deviation of the total population (.768). In strain 35, the difference was

.209 or 35.2 per cent of the standard deviation of the total population (.593). The proportion of the total variance due to sex is given by the ratio of the square of the half difference to the total variance, or one fourth the square of the above fractions. It appears that 2.5 per cent of the variance in the random bred stock and 3.1 per cent in strain 35 was due to sex. The analysis into genetic and nongenetic portions applies of course to the 98–97 per cent not due to sex.

The littermate correlation in strain 35 (+.103) can be taken as indicating the portion of the variance (within each sex) which is due to environment common to littermates. This is on the assumption that the parent-offspring correlation (+.024) can be ignored. If the latter is accepted, the estimate is reduced to +.079. The figures from the other inbred strains confirm the existence of an effect of the order of these figures as does the difference (+.091) between littermate and parent-offspring correlations in the control stock. It is indicated that the greater portion of the variance (89.7 per cent in strain 35) is due to environmental factors so local in incidence that they do not affect littermates alike.

TABLE 17

Mean percentages of white in males and females of strain 35, at two periods, in relation to age of mother

AGE OF MOTHER (MONTHS)	STRAIN 35 (1916–24)				STRAIN 35 D (1926–34)			
	MALES		FEMALES		MALES		FEMALES	
	NO.	AV.	NO.	AV.	NO.	AV	NO.	AV.
3– 5	182	56 3	153	60.5	25	56 0	31	65 0
6– 8	195	59.5	187	67.6	45	67 8	41	70.2
9–11	152	60.6	160	66.5	40	71 7	21	78 1
12 14	150	61 3	124	70.6	33	69 8	30	76 3
15–20	174	63 2	149	69.6	27	61.9	34	74.7
21- 46	138	66 9	144	73.3	23	78.2	25	77.6
Total	**991**	**61.1**	**917**	**67.8**	**193**	**67.8**	**182**	**73.1**

The only direct evidence on the nature of the environmental factors common to littermates is given by tabulations of grade of spotting in relation to age of mother. Such tabulations have been made in strains 35, 35 D, 2 and 13. In the last two cases no significant relations were found, but the highly asymmetrical distributions banked up against the limit of 100 per cent white make these unfavorable material for detecting slight differences. The results in 35 and 35 D are shown in table 17. In strain 35, there is a fairly regular increase in percentage of white with increasing age of the parents (usually littermates) at a rate of 0.68 per cent per month

TABLE 18

Joint frequencies of parent and offspring in inbred strain 35. Correlation (broad categories) +.026

PARENT \ OFFSPRING GRADE OF SPOTTING	X-	3-	5-	7-	9-	11-	13-	15-	17-	19-	TOTAL
19-20	—	1	5	6	8	7	22	18	10	4	81
17-18	1	7	28	22	51	68	82	73	53	14	399
15-16	6	8	24	43	38	80	97	96	57	17	466
13-14	2	7	25	35	74	90	103	121	72	14	543
11-12	6	8	22	44	59	102	105	99	77	21	543
9-10	3	7	19	24	34	60	66	58	62	10	343
7-8	3	6	14	29	34	64	51	63	45	8	317
5-6	—	2	6	13	22	15	28	20	16	1	123
3-4	1	—	—	2	1	1	1	1	1	—	8
X-2	2	—	1	2	5	5	7	3	3	1	29
Total	24	46	144	220	326	492	562	552	396	90	2852

up to 15 months of age. This factor determines about 3.6 per cent of the total variance and thus approximately half of that common to littermates. In 35 D, the results are more irregular as expected from the smaller numbers but the correlation of +.252 ± .069 for females, +.203 ± .069 for males give clear evidence of significance (the average +.227 ± .049 is 4.6 times its standard error). From the square of this figure it appears that about 5 per cent of the variance (untransformed scale) was due to this factor. This would be slightly reduced by transformation of the scale. The regression, 0.76 percent white per month (up to 15 months) agrees reasonably well with the earlier data. It should be noted that in case (35 D) the effect is definitely one of age of mother, since most matings were intentionally

TABLE 19

Comparison of correlations obtained by the broad category method with the averages of those given in tables 15 and 16.

STRAIN	PARENT-OFFSPRING NO.	PARENT-OFFSPRING CORRELATION TABLES 15, 16	PARENT-OFFSPRING CORRELATION BROAD CAT.	LITTERMATES NO.	LITTERMATES CORRELATION TABLES 15, 16	LITTERMATES CORRELATION BROAD CAT.	SIBS, DIFFERENT LITTERS NO.	SIBS, DIFFERENT LITTERS CORRELATION TABLE 15	SIBS, DIFFERENT LITTERS CORRELATION BROAD CAT.
35	2852	+.024	+.026	1367	+.103	+.075			
35D	771	+.026	+.020	360	+.115	+.083	1046	−.048	−.045
2	1680	+.064	+.059	766	+.078	+.101			
13	1846	+.009	+.008	914	+.077	+.090			
13E	1484	−.050	−.066	712	+.018	+.005			
Av. 5 inbred strains	8633	+.016	+.012	4119	+.079	+.072			
Random bred-stock	3881	+.191	+.199	2080	+.282	+.260			

made between males and females of widely different ages (WRIGHT 1935, p. 526).

It has seemed desirable to check the parent-offspring and littermate correlations by a different method of allowing for the distortion of the scale. For this purpose the tabulations by sex were combined into single tables and correlations calculated by PEARSON'S method for broad categories. The parent-offspring distribution for strain 35 is shown in table 18; those for littermates in this stock and those in the random bred stock have been deposited with "Genetics." In the broad category method, the mean of each class on a hypothetical normal scale, is calculated by the formula $x=\frac{z_1-z_2}{q_1-q_2}$ where q_1 and q_2 are the observed tail frequencies (fractions of 1) cut off by the class limits and z_1 and z_2 are the theoretical ordinates of the unit normal curve at these points. The approximate correlation is given by the formula $r_{x_1x_2}=\frac{\Sigma x_1x_2f}{n\sigma_{x_1}{}^2\sigma_{x_2}{}^2}$. The results are given in table 19. Comparisons are made with the averages of the correlations for separate sexes from tables 15 and 16. The latter might be expected to be slightly larger because of the elimination of sex differences but the different scales prevent exact comparison. On the whole the differences are unimportant.

TABLE 20

Deviations of class limits from the median in the random bred stock and in strain 35 in terms of their standard deviations assuming normality See fig 1

CLASS LIMIT (% WHITE)	RANDOM BRED STOCK		STRAIN 35	
	100 q	$\frac{x}{\sigma}$	100 q	$\frac{x}{\sigma}$
0	0	−∞	0	−∞
12.5	1.37	−2.206	0.84	−2.391
22.5	3.46	−1.817	2.45	−1.969
32.5	7.09	−1.469	7.50	−1.439
42.5	13.17	−1.118	15.21	−1.027
52.5	21.62	−.785	26.64	−.624
62.5	32.88	−.443	43.89	−.154
72.5	47.90	−.053	63.60	+.348
82.5	66.09	+.415	83.95	+.952
92.5	80.98	+.877	96.84	+1.858
100	100.00	+∞	100.00	+∞

In this method, the scale is adjusted in each case so as to yield a corrected standard deviation of 1. Thus each distribution yields a separate scale. The transformed location of each class limit of the observed scale can be found by taking $X/\sigma=prf^{-1}(q-.50)$. This is done for the random bred stock and for strain 35 in table 20 and the results plotted against each other in figure 1. With the exception of the boundary between classes 18 and 19 (92.5 per cent white) the values fall rather closely along a straight line. Taking the values at 42.5 and 82.5 as those closest to the standard deviations, considering both scales (fig. 1) we see that a scale interval of 1.533σ in the random bred stock corresponds to one of 1.979σ for strain 35. The corrected standard deviation of strain 35 is thus 77.5 per cent $= \frac{1.533}{1.979}$ of that of the random bred stock and its variance is 60.1 per cent $(=.775^2)$

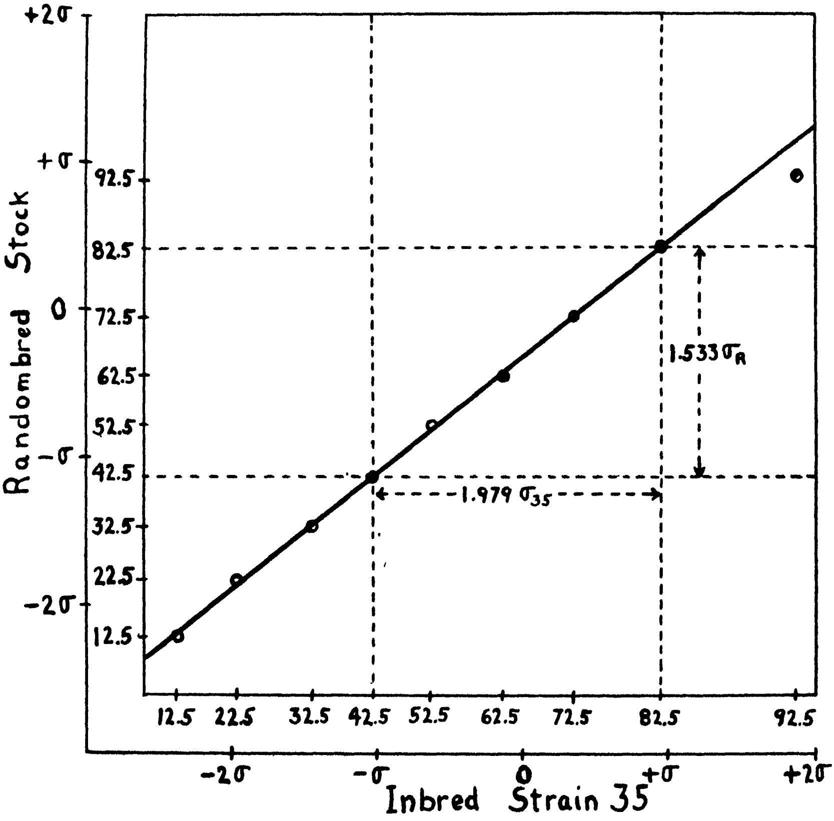

FIGURE 1. Transformation of class limits to give normal distribution of class frequencies in random bred stock plotted against similar transformation for inbred strain 35. The range between 42.5% white and 82.5% white includes 1.979σ in the inbred strain but 1.533σ in the random breds. The variance of the inbred stock is thus 60.1% $(=1.533^2/1.979^2)$ of that of the random bred stock on a scale based on these two points.

of that of the random bred stock. Since the correlation between parent and offspring in the random bred stock was +.199 by the broad category method, the genetic variance (assuming no dominance or epistasis) should be 39.8 per cent and the variance to be expected in an isogenic stock should be 60.2 per cent of that in the random bred stock, or almost exactly the value actually found in strain 35 by the above method. The agreement with results from the previous method is as close as can be expected. There can be little or no dominance or epistasis among the minor factors which affect spotting.

Putting all of these results together we reach the following approximate analysis of variance in the random bred stock and an isogenic inbred strain.

	Isogenic Inbred Strain	Random Bred Stock
Heredity	0	40
Sex	3	2
Environment		
Age of mother	4	6
Other factors common to littermates	4	
Factors not common to littermates	89	52
	100	100

DISCUSSION

The results fall into a consistent picture. Four classes of factors are indicated.

First is a major pair of alleles, *S,s*. *SS* is usually self but under exceptional conditions shows traces of white up to a small shoulder belt; *Ss* may be self colored or any grade of spotted up to at least 95 per cent white; *ss* is usually well marked with white, often self white, but may have only a trace of white and perhaps may be completely self-colored under very unusual conditions.

Second is a multiplicity of minor genetic factors with additive effects (little or no dominance or epistasis). The median grade of *ss* can be shifted from about 10 to 97 per cent white by the appropriate combination. The median grade of *Ss* may similarly be shifted from self color to about 30 percent white. Finally a little white may appear in *SS* with an extreme array of white modifiers.

Third, there is an enormous amount of non-genetic variability responsible for a range extending from a trace of white to 100 per cent white in isogenic strains whose median grade is near 50 per cent. This can be subdivided into a small portion composed of factors common to littermates, in which is included an effect of age of mother in at least one stock, and a large portion not common to littermates and hence to be interpreted as due to developmental accidents. In a typical random bred stock, all *ss*, about 58 per cent of the variance was due to non-genetic factors, including only 6 per cent common to littermates.

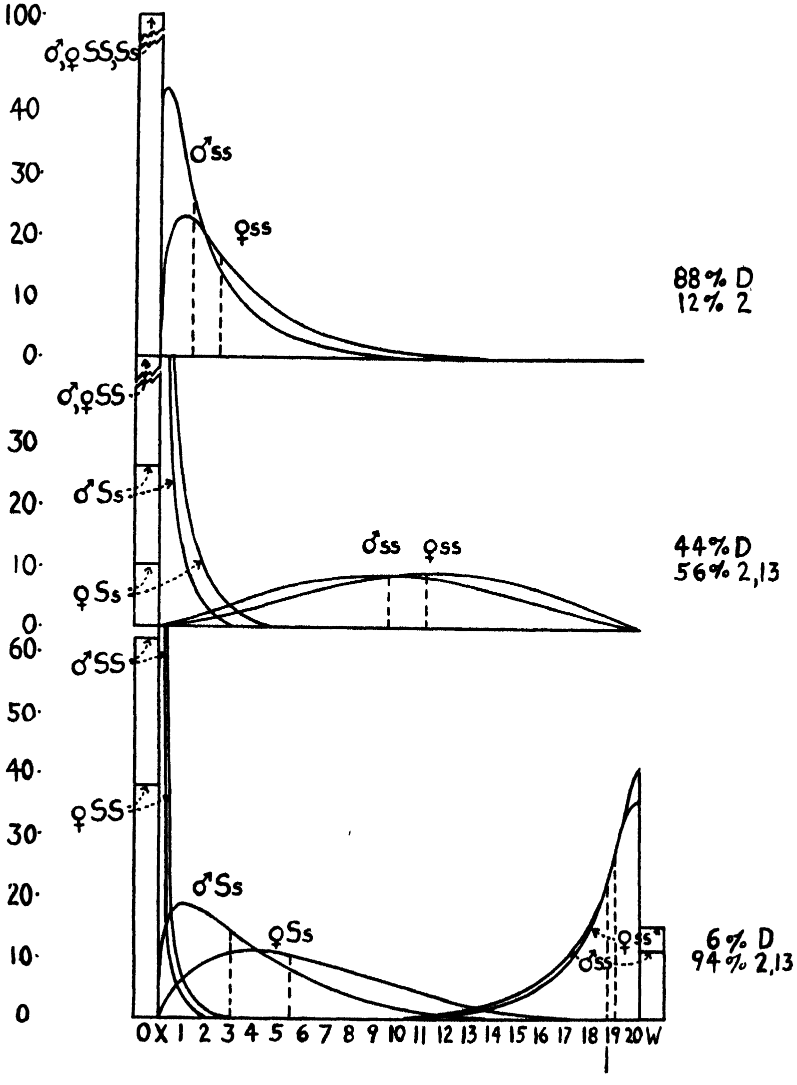

FIGURE 2. Distributions of *SS*, *Ss*, and *ss* according to sex and residual heredity. The abscissas are the classes used in grading. The ordinates are the fitted percentage frequencies in these classes, doubled in the cases of X and 20 to allow for the fact that their ranges are only of half width. Classes O and W are arbitrarily assigned full class ranges. Smooth curves are fitted by the formula $prf^{-1}[\sum f - .50] = 1/.57\,[prf^{-1}(x - .50) - prf^{-1}(M - .50)]$ where prf^{-1} is the inverse probability function, $\sum f$ is the running sum of the fractional frequencies, .57 is the standard deviation (on the transformed scale) of an isogenic stock and M is the median of the distribution to be fitted. The class limits and medians are modified slightly by replacing x by the function $.98x + .0025$ in order to give a class of solid color 0%–.25%) and a class of solid white (98.25%–100%). The medians in the upper series are from 7/8*D* table 7, those in the middle series from (7/8*D*×2), table 7 in the case of *ss* and from (7/8*D*×13), table 8, in the case of *Ss*. The medians in the bottom figure are based on backcrosses (and derivatives) with 7/8 or more of strain 13 (*SS* table 10: *Ss*, *ss* table 9). The distributions so fitted all agree reasonably well with the observed distributions.

Fourth is a sex difference, females having slightly more white than males in all strains, the difference accounting for 2 or 3 per cent of the total variance.

The effects of these four classes of factors are illustrated in figure 2. The variability of males and females of *SS*, *Ss* and *ss*, due to non-genetic factors are shown at the top on a background of modifiers suppressing white, at the bottom with modifiers favoring white, and in the middle with a more typical, intermediate combination of modifiers. The sex difference appears to be greatest in stocks with somewhat less than 50% white in the coat on the average.

We have finally to consider the relation of these results to those of other authors.

Our pair, *S*,*s*, is doubtless the same as IBSEN'S *S*,*s* although IBSEN refers to *S* as dominant. IBSEN'S other factors *Fa*, *fa*; *Na*, *na*, obviously cannot be used in the interpretation of our results. Judgment as to whether the enormous amount of non-genetic variability of all of our stocks is largely absent from his stocks must await publication of his data.

PICTET'S 4-factor hypothesis also is obviously inapplicable to our data. In our data there is clearly just one major factor with effects on both head and trunk; *ss* typically has much white on both head and trunk, in *Ss* both are reduced while *SS* extracted after many generations of back-crossing to a stock in which both head and trunk are almost wholly white, is usually completely self. The correlation between head and trunk is not perfect, but neither is the correlation between any two areas which could be named, for example, left and right ears, left and right fore feet, shoulder and loin (cf. ILJIN 1928). PICTET has presented no data which justify the conclusion that his stock differs in these respects.

PICTET'S most extensive published data refer to the trunk pattern. How far is our interpretation applicable? It is not possible to give a certain answer. However, it seems possible that the dominant and recessive spotting factors which he found in his two original sets of matings may have been the same factor, *s*, associated with different arrays of modifiers as in our experiments involving 7/8 blood strain 13 and 7/8 blood strain D respectively. With respect to his later generations, derived from crosses of the two foundation stocks, there are so many possible genotypes for every phenotype in both hypotheses and such a wide range of phenotypes for each genotype in ours that it seems not unlikely either could be made to fit. It would require experiments with isogenic stocks to discriminate between them.

Whether there is any non-genetic variability in his stock, that is, a wide range of phenotypes for each genotype, is not considered by PICTET. But the irregularity in localization of spots and frequent asymmetry which seem to have characterized his stock as well as ours make the existence of such variability probable. If a type of spotting is ever discovered in the guinea pig, which is not subject to non-genetic variability to an important extent, it will probably show something of the orderliness of pattern of hooded rats or Dutch rabbits.

SUMMARY

Analysis of the results of crosses among a considerable number of closely inbred strains of guinea pigs, supplemented by biometric analysis of variability and correlations in random bred and inbred stocks indicate 4 classes of factors as affecting white spotting:

(1) a major pair of alleles *S*,*s* in which *S* (tending toward self) is usually incompletely dominant (statistically) over *s* (tending toward white),

(2) a multiplicity of genes with individually small effects, additive on a suitably transformed scale (no dominance or epistasis),

(3) an enormous amount of non-hereditary variability, not common for the most part even to littermates, but including minor effects of common factors (for example increasing white in young with increasing age of mother),

(4) a sex difference, females having slightly more white than males on the average in all strains.

The interpretations which have been put on white spotting by other recent authors, who have largely ignored the possibility of non-hereditary variability, are shown to be inapplicable to the results described here.

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GENETICAL AND CYTOLOGICAL STUDIES OF A DEFICIENCY (NOTOPLEURAL) IN THE SECOND CHROMOSOME OF DROSOPHILA MELANOGASTER

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THE conclusion that two dominant venation mutants (Plexate and Plexate^2) and two minutes (Minute-l and Minute-4) are due to losses from the gene-string—that is, to deficiency—was drawn several years ago on the basis of genetic data and was later confirmed by examination of the salivary chromosomes. Several other genetic deficiencies, notably the Notches, have been similarly located through salivary analysis, by PAINTER, MACKENSEN, MULLER, PROKOFYEVA, DEMEREC and other workers. All of the dominants, especially those which are lethal when homozygous, require checking for chromosomal rearrangements, such as deficiency, duplication, translocation and inversion. An important goal in this work is the establishment of close correspondences between the loci on the genetic maps, deduced from linkage studies, and the particular chromosome localities, deduced from study of the transverse bands which form a diversified series along the salivary chromosomes.

ORIGIN AND CHARACTERISTICS OF NOTOPLEURAL

In the balanced stock of Stubble/*C3,l3a* it was observed by SKOOG (Feb. 20, 1933) that a small percentage of both males and females had shorter, wider, blunter wings, with venation irregularly thickened or branched in a few places. In some individuals there was a break in the posterior crossvein. A Stubble male showing this spontaneous mutant character was outcrossed to Curly (Exp. 610). In F_1 the mutant wing type appeared in about half the progeny of both sexes, hence is an autosomal dominant. Upon inbreeding the F_1 ("Mutant"/Cy; Sb/3^+) flies, it was found that all F_2 flies showed both Curly and the "Mutant"; hence "Mutant" has its locus in Chromosome II, and is completely lethal when homozygous. A balanced stock of "Mutant"/Cy, freed of Stubble, was obtained in F_3 and kept. When the "Mutant" was thus separated from Stubble it was found to have itself somewhat shortened bristles, especially the humerals, notopleurals and pretarsals (see fig. 1, heterozygous female). The shortened bristles proved more reliable for classification than the venation and other wing characters; hence the "Mutant" was renamed Notopleural, with symbol *Np*. There are several other alterations characteristic of the Notopleural type, of which the most obvious is the straggly arrangement of hairs on the thorax. All the Notopleural characteristics are somewhat more extreme in females, and at temperatures above 25°C. A majority of *Np* females are completely sterile, while the fertile ones give progenies reduced to half or even to a small fraction of the normal number. The males are fertile and productive. Viability of *Np* is erratic, from practically normal to half-normal. Emergence is delayed somewhat by slow development, but the resulting flies are large and strong in appearance. (In relative valuation the mutant is classed in Rank-4.)

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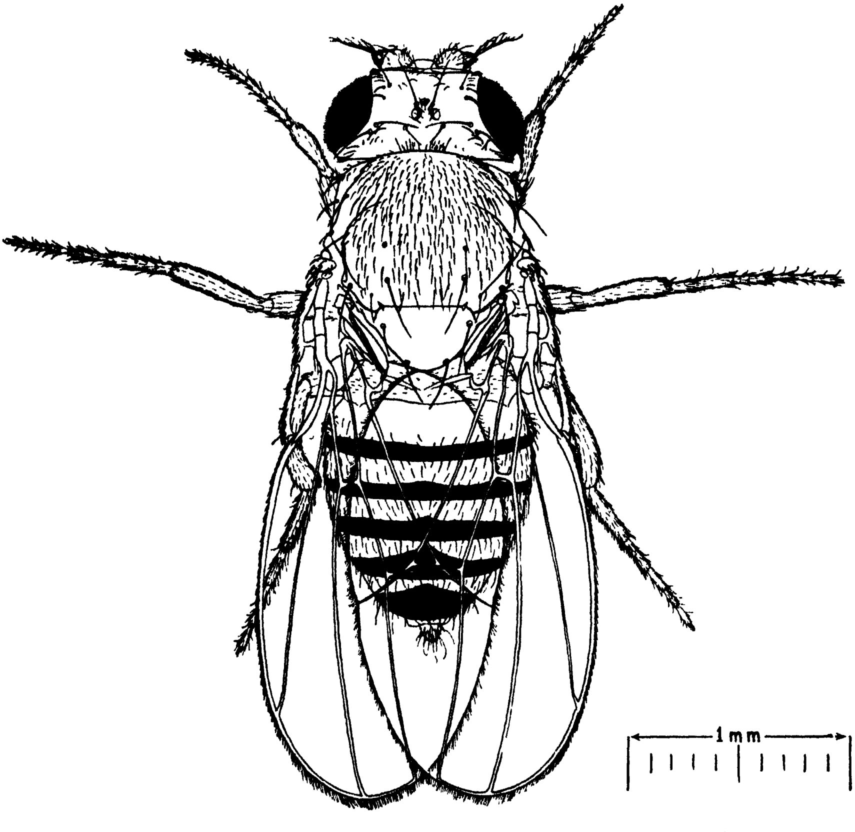

FIGURE 1. Notopleural female heterozygote (E. M. Wallace, Del.)

LINKAGE RELATIONS AND LOCUS OF NOTOPLEURAL

Female testcrosses of Notopleural with brown (104.5) and speck (107.0) gave free recombinations, showing that the locus of *Np* is far to the left of those of *bw* and *sp* (Exp. 610A; 3 fertile, 25 sterile cultures; SKOOG; 117–19; June 7, 1933):

Np	*bw sp*	*Np bw sp*	+	*Np sp*	*bw*	n	*Np-bw R*	*bw-sp R*
105	119	57	66	2	3	352	35.0	1.4%

Next, it was found that the *Np* locus is to the right of Sternopleural (22.0) and of black (48.5) (Exp. 610B; 6 fertile cultures; BRIDGES; 20,370–76; July 19, 1933):

Sp b	*Np*	*Sp Np*	*b*	*Sp Np b*	+	n	*Sp-b R*	*b-Np R*
40	33	9	4	3	3	92	14.1%	6.5%

Further *b* +/+ *Np* linkage data were obtained (Exp. 610C; 3 fertile cultures; BRIDGES; 20,383–85; Aug. 1, 1933):

b	*Np*	*b Np*	+	n	*b-Np R*
106	89	7	12	214	8.9%

Testcrosses of the type *b Np* +/+ + L^2 (Exp. 610D; 14 fertile; BRIDGES; 20,440 ff; Aug. 29, 1933) gave:

b Np	L^2	*b* L^2	*Np*	*b Np* L^2	+	*b*	*Np* L^2	n	*R-1*	*R-2*
468	753	94	76	68	165	19	11	1654	12.1	15.9

From the three foregoing tests in which black and Notopleural were involved, 1986 flies gave 11.4 as the mean percentage of *b-Np* recombinations, with the locus of *Np* between *b* and L^2, at about 60 (48.5+11.5).

More precise localization, as well as more accurate determination of possible crossing over reduction, was made by use of the nearer loci purple (54.5) and engrailed (62.0) in female testcrosses with Notopleural (Exp. 610G; 10 fertile cultures, 3 ♀ ♀ each; SKOOG; 216 ff; Oct. 5, 1933):

pr en	*Np*	*pr Np*	*en*	*pr*	*Np en*	+	n	*R-1*	*R-2*
392	342	13	31	9	2	1	790	5.7%	1.5%

From this testcross the *pr-en* crossing over was 7.2, as compared with the standard 7.5; evidently no marked reduction of crossing over was produced by the Notopleural mutation. The locus of Notopleural was indicated as 1.5 to the left of engrailed (62.0), hence at 60.5, in agreement with the position as deduced from the *b-Np* data.

At this stage cytological examination of the salivary chromosomes (see below) confirmed the hypothesis that Notopleural is due to a deficiency. Since the deficiency is nearly 5 per cent of the length of the right arm of chromosome II, a local reduction in crossing over is expected, hence more precise tests of the linkages were carried out by LI to check this relation.

There are two good characters with loci between purple and engrailed, namely, cinnabar eye-color (57.8) and bloated wing (59.0). The character bloated (found by P. T. IVES, June 26, 1933), although fairly good (RK2), has been little utilized. For closer study two new stocks were required, namely, *cn en* and *blo en*. To secure *cn en*, *pr cn* was crossed to *cn*, and the F_1+ ♀ ♀ backcrossed to *pr en* ♂ ♂ (Exp. 610J; 7 cultures; L1; Jan. 10, 1936):

pr en	+	*pr*	*en*	n	*pr-en R*
758	851	103	140	1852	13.1%

Some of the not-*pr en* flies (140) were crossed together in mass cultures. In the next generation they produced *cn en* flies, which were bred together for the required stock.

Similarly, the female testcrosses of *pr*+*en*/+*blo*+ ♀ ×*pr*+*en* ♂ ♂ gave *pr-en* recombination data and in due course a stock of *blo en* (Exp. 610K; 9 cultures; L1; Jan. 10, 1936):

pr en	+	*pr*	*en*	n	*pr-en R*
1365	1511	125	148	3149	8.8%

The above two control experiments gave diverse values of 8.8 and 13.1 per cent of recombination for *pr-en*, as compared with standard 7.5. Also, great fluctuation occurred from culture to culture within both tests. Such high variability is rather characteristic of the central regions of chromosomes II and III, and makes comparisons of data of far less value than for other regions.

As a check on the normality of the *cn en* stock, and as a control against the anticipated results with Notopleural, a rather extensive testcross of *cn en*/Oregon-Roseburg (Ore-R) ♀ ♀ was carried out (Exp. 610L; 26 cultures; L1; Feb. 13, 1936):

cn en	+	*cn*	*en*	n	*cn-en R*
4445	4646	322	324	9737	6.6%

A similar control of *blo en*/Ore-R ♀ ×*blo en* ♂ ♂ (Exp. 610M; 30 cultures; L1; March 1, 1936) gave:

blo en	+	*blo*	*en*	n	*blo-en R*
2772	4793	142	199	7906	4.3%

Finally, a linkage experiment involving *cn en* and Notopleural was carried out (Exp. 610N; 34 fertile cultures; L1; Feb. 14, 1936):

cn en	*Np*	*cn Np*	*en*	*cn*	*Np en*	n	*R-1*	*R-2*
2931	2298	16	23	110	63	5441	0.7%	3.2%

The similar *blo*+*en*/+ *Np*+ testcrosses (Exp. 610P; 36 fertile cultures; L1; March 1, 1936) gave:

blo en	*Np*	*blo Np*	*en*	*blo*	*Np en*	n	*R-1*	*R-2*
450	619	0	0	10	19	1098	0.0%	2.6%

These two experiments (*cn en/Np* and *blo cn/Np*) and their controls (*cn en*/Ore-R and *blo en*/Ore-R) show that there is very probably a reduction of crossing over due to the deficiency. There are two measures of the amount of this reduction, namely: 6.6 − 3.9 = 2.7 and 4.3 − 2.6 = 1.7. Supplementary to these, we might give the calculations 4.3 − 2.9 = 1.4 (where 2.9 is the mean value for the three experiments giving *Np-en* data) and 10.3 − 7.2 = 3.1 (where 10.3 is the mean from the two *pr-en* controls and 7.2 is SKOOG's value for *pr-en* as modified by *Np*). The weighted mean from these four measures is about 2.0. But since all the experiments on which this value is based gave approximately 30 per cent more crossing over than expected from the standard map, a value of 1.5 would be more comparable with standard results as the amount of reduction due to Notopleural. Since the genetic length of the right limb of Chromosome II is about 52 units, and the salivary deficiency includes 4.4 percent of the right limb (see below), we might expect that the deficiency would make about 2.3 units reduction, if crossing over is equally distributed. But the general correlation of the linkage maps with the salivary maps shows that crossing over is only about half as free in this particular region as in the right limb as a whole. Hence, only about 1.2 would be the reduction expected, in agreement with the value 1.5 deduced from the experiments. While crossing over is probably entirely eliminated from within the deficient section, it may also be reduced somewhat in the immediate vicinity.

The locus of Notopleural cannot be definitely ascertained by linkage if there is crossing over reduction in the vicinity. If the 1.5 represents the length of the deficient section, the location should be described as extending between two points. These might be approximated at 1.8 to the left of engrailed, or at 60.2, for the right end, and 1.5 further to the left, or at 58.7, for the left end.

CHECK OF NOTOPLEURAL DEFICIENCY FOR POSSIBLE INCLUDED LOCI

In the general neighborhood of Notopleural (58.7–60.2) ±, the standard map (D.I.S. 3) showed the following mutants: staroid (58 ±), bloated (59 ±), engrailed (62.0), upward (62.5 ±) chaetelle (63 ±), Baroid (65 ±), Xasta (68 ±) and Abnormal-wing (65 ± 5). The mutants Baroid, Xasta and Abnormal-wing are associated with translocations. Tests showed that all of these loci are outside the deficiency. The closest neighbor is bloated to the left, and this agrees with the observation that *blo en/Np* gave no *blo-Np* recombinations. A more probable position of *blo* is thus at about 58.5.

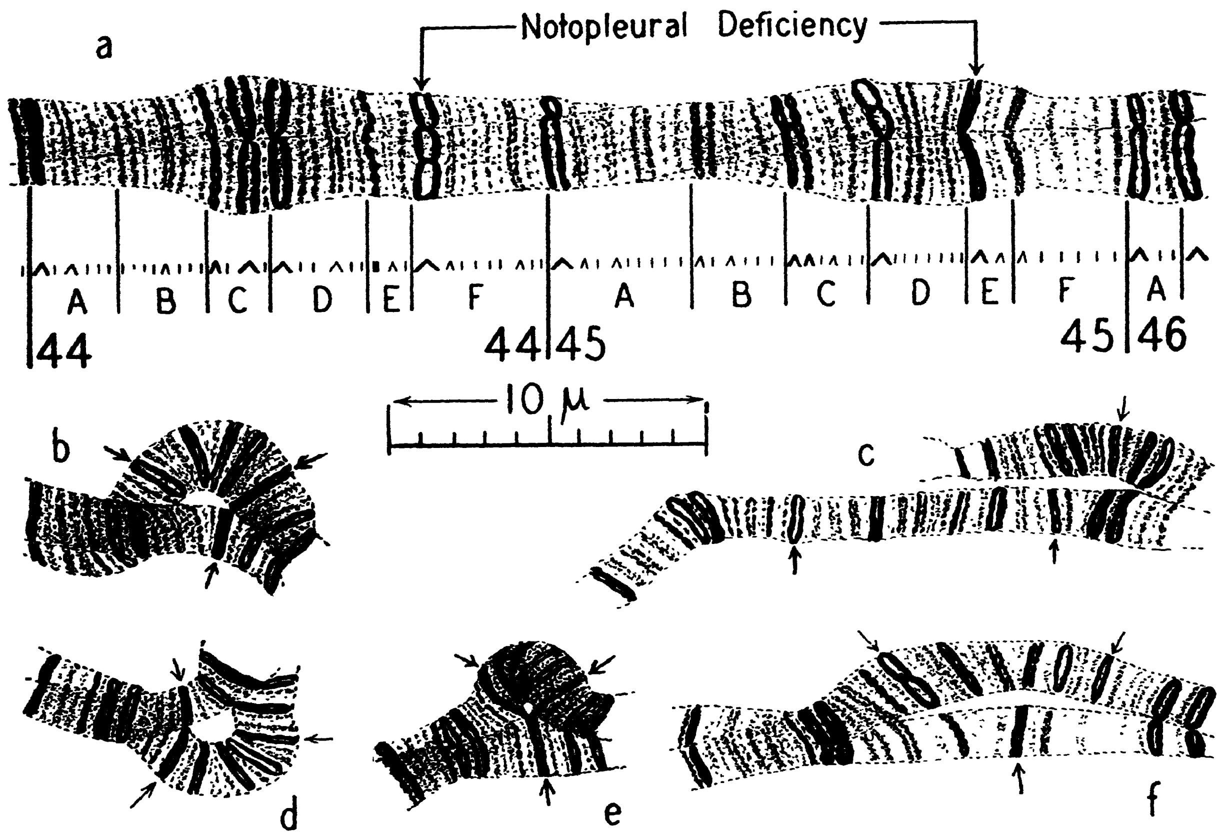

FIGURE 2. Salivary chromosomes of Notopleural heterozygotes. a. Revised normal map of sections 44 and 45 in 2R, with the Notopleural deficiency indicated. b. Synapsis at right limit. c. Non-synapsed strands, both lax. d. Synapsed at left limit. e. Synapsed at both limits. f. Well stretched, non-synapsed deficient strand. (C. B. Bridges, Del.)

SALIVARY ANALYSIS OF NOTOPLEURAL

The first confirmation of the hypothesis that Notopleural is a deficiency was made (Nov. 22, 1935) by LI, who observed a "buckle" and loop at about section 45 in the right limb of Chromosome II (BRIDGES 1935). For a more exact study, numerous permanent preparations of salivary chromosomes of Notopleural/Ore-R females were made. Studies of the normal morphology of sections 44 and 45 were carried out by BRIDGES on Oregon R and numerous other strains. By averaging the measurements and spacings of camera lucida drawings of the five most favorable stretched specimens, a revised map of this region was made (fig. 2a). Easy landmarks to the left of the deficiency are the three heavy doublets in 44 CD, while equally striking markers to the right are the two heavy doublets at the beginning of 46.

Study of the banding (fig. 2c, and especially 2f) showed that the Notopleural chromosome is normal through 44E and from 45E on. But at the rejunction is a doublet not exactly matching any band in the normal chromosome, being less heavy and less separated than the heavy doublet beginning 44F, and heavier than the close doublet beginning 45E. Study of the synapsis relations showed this anomalous band sometimes in synapsis with the 45E1 doublet (fig. 2b) but more frequently in synapsis with 44F1 doublet (fig. 2d) and occasionally with both at once (fig. 2e). Evidently both breaks of the deficiency split through the halves of doublets. The normal map shows 50 distinguishable transverse elements between the breaks of the Notopleural deficiency.

The interpretation to which we incline is that normal 44F1 doublet is a "repeat" (BRIDGES 1935) of two identical bands and that normal 45E1 doublet is similarly a case of identical twin bands. The new band ($\frac{1}{2}$ 44F1 doublet+$\frac{1}{2}$ 45E1 doublet) shows close union of the two parts, suggesting allelism. On this view the two normal doublets would be alleles, one derived from the other, or both modifications of a previous common ancestor.

It is suggested that "repeats" offer a generalized, and perhaps the most frequent, mechanism for further steps in rearrangement—either translocations, inversions or deficiencies—through a preliminary synapsis of homologous or allelic bands or sections, which are carried in separate chromosomes or localities, and subsequent crossing over to give the new configuration. Striking evidence for this general view will be presented in studies of certain other rearrangements.

SUMMARY

The dominant mutant "Notopleural" (symbol *Np*) was found as a spontaneous occurrence by ELEANOR NICHOLS SKOOG, February 20, 1933.

It is characterized by numerous slight departures from the wild type, especially by shortened notopleural, humeral and pretarsal bristles, by straggly microchaetae, by blunter wings with somewhat thickened and branched venation, by low production of eggs by *Np* females and by erratic mortality. The homozygote is completely lethal.

Linkage tests showed the locus to be in the right limb of Chromosome II, between the locus of bloated (58.5) and engrailed (62.0). Crossing over is locally reduced in the presence of *Np* by about 1.5 units. Since *Np* is a deficiency (see below) its limits on the normal map are about 58.7 to 60.2.

Salivary analysis shows a deficiency of 50 recognized bands. Both breaks are between the halves of doublets and the deficient chromosome shows a new doublet composed of the left half of 44E1 heavy doublet and the right half of 45E1 doublet which is slightly less heavy.

It is suggested that these two doublets are homologous, though one or both may have been altered by mutation from their common ancestor. The occurrence of such homologous "repeats" gives a reason for the exchange points or breaks of translocations, inversions and deficiencies coming at particular favored places.

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GENETIC MAPS OF THE AUTOSOMES IN DROSOPHILA PSEUDOOBSCURA

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INTRODUCTION

DROSOPHILA PSEUDOOBSCURA is of considerable interest as an object for the study of a number of genetic problems, especially those connected with the hybrid sterility and the methods of race and species formation. As a basis for these studies it is necessary to work out the genetic and the cytological maps of the chromosomes of this species. The present paper deals with the genetic maps of its autosomes.

MATERIALS AND METHODS

Since the two races of *Drosophila pseudoobscura* are different in gene arrangement—they differ in an inversion in each limb of the X chromosome and an inversion in the second chromosome (TAN 1935a,b, KOLLER 1936)—and since strains of the same race from different geographic origins may also differ in gene arrangement, it becomes imperative for the purpose of this work to use always a definite standard strain. Race A strain from Georgetown, Texas, was chosen.

All mutant stocks of race A *D. pseudoobscura* kept in this laboratory were used. The author is indebted to Miss BEERS of the University of Southern California for the use of some mutants she discovered. Some mutant stocks were sent here from the Genetics laboratory of Edinburgh University, Scotland, through the kindness of Drs. KOLLER and CREW, to whom the author wishes to express his appreciation. In the course of the investigation a few new mutants were discovered by the author and some of them proved valuable because of their favorable locations. Prof. STURTEVANT kindly let me use some of the new mutants he had found.

For linkage experiments all cultures were raised in an incubator at 25 ± .5°C.

DESCRIPTION AND LOCALIZATION OF MUTANT GENES

Since the present paper is confined to the autosomes of *D. pseudoobscura*, only the autosomal mutants will be briefly described below. In each chromosome mutants will be described more or less in the order of their discovery.

The second chromosome

Bare (*Ba*). Bare was discovered by STURTEVANT (1934) in race A. It is characteized by the shortening of all the macrochaetae. The character can be easily distinguished from the wild type, and the viability and productivity of the heterozygous flies are excellent. Homozygous Bare flies are occasionally viable.

Smoky (*Sm*). Smoky, another dominant gene in this chromosome, was found by Miss BEERS (1934) in race B. It has been successfully transferred to race A. The mutant gene causes the thickening and branching of wing veins, especially the second longitudinal vein. The minimum expression is the formation of a small delta-like structure at the distal end of the second longitudinal vein. It is invariably lethal when homozygous. Smoky also has the effect of roughening the surface of the eyes. By crossing to Bare and backcrossing the F_1 Bare Smoky females to wild type males, it was found that the crossing over frequency between the two exceeds 40 per cent.

TABLE 1

Two point linkage experiments.

LOCI	TYPE OF CROSS	NON-CROSSOVERS		CROSSOVERS		TOTAL
		Chromosome II				
bi Ba	*bi*×*Ba*	135	145	21	7	308
		Chromosome III				
Sc cv	*Sc*×*cv*	181	175	141	158	655
		Chromosome IV				
in j	*in j*×+	199	177	59	26	461
in j	+×*in j*	354	341	54	73	822
in Cy	*in*×*Cy*	128	104	117	119	468
j tg^3	+×*j* tg^3	149	157	103	112	521
j Cy	*Cy*×*j*	886		235		1121
tg^3 *Cy*	tg^3 *Cy*×+	261	288	30	54	633

Glass (*gl*). The recessive mutant glass was discovered and described by CREW and LAMY (1935a). The eye is reduced in size and surrounded by a smooth colorless rim. The pigment in the central space appears to be greatly reduced in amount and leaves only a pinkish or reddish hue. The character can be easily recognized with the naked eye. The viability and fertility are about as good as in wild type. In a cross between *Sm Ba/gl* ♀ ×*gl* ♂, the result (table 2) shows crossing over values of 42.0 per cent between Bare and Smoky, 18.9 per cent between Bare and glass, and 46.3 per cent between Smoky and glass. Since the smallest number of flies were represented by Bare-glass and Smoky classes, which must be double crossovers, the sequence of the three genes in the chromosome must be Smoky-Bare-glass, Bare being nearer to glass than to Smoky.

Bithorax (*bi*). The gene bithorax was first reported by CREW and LAMY (1934b) as an autosomal recessive mutant, causing an enlargement of the balancers. In extreme cases, the balancers may take the form of wing-like organs. The mutant flies are relatively weak and their viability is low. Sometimes the character may overlap normal.

DONALD (1936) described the mutant in detail and reported that the locus of bithorax is 25 units from glass. My data from the cross *bi gl/Sm Ba* ♀ to *bi gl* ♂ (table 3) show that bithorax is 28.9 units from glass. Between glass and bithorax lies the gene Bare, which gives 21.2 per cent of recombinations with glass on one side and 9.7 per cent with bithorax on the other (table 4).

TABLE 2

Three point linkage experiments.

LOCI	TYPE OF CROSS	NON-CROSSOVERS		SINGLE CROSSOVERS				DOUBLE C.O		TOTAL
				REGION 1		REGION 2		1, 2		
				Chromosome II						
Sm p ps	*Sm p ps*×+	134	59	50	95	81	45	39	39	542
Sm Ba gl	*gl*×*Sm Ba*	90	164	137	52	36	27	20	20	546
bi p Ba	*bi p*×*Ba*	226	206	20	9	7	5	4	4	481
bi p ps	*bi p ps*×+	223	138	10	9	165	89	7	4	645
bi gl ps	+×*bi gl ps*	119	21	44	21	24	10	6	5	250
				Chromosome III						
or Bl Sc	*Bl Sc*×*or*	160	175	19	13	34	39	1	1	442
or ab pr	*or ab pr*×+	251	146	9	29	182	94	7	18	736
or Sc pr	*or Sc pr*×+	74	45	17	19	23	17	5	6	206
				Chromosome IV						
in j tg^3	*in j* tg^3×+	172	90	21	59	70	104	13	5	534
tg^3 *Cy Ro*	*Ro*×tg^3 *Cy*	157	160	19	11	5	2	0	0	354

Pink (*p*). The gene for pink eye color was found in a cross of the La Grande-2 strain to orange Scute purple by STURTEVANT in 1934. It is recessive and located near Bare. Pink itself is variable and sometimes overlaps wild type. However, in combination with orange, a third chromosome gene, it can be easily classified. As a matter of fact, orange alone gives a bright red eye color (like vermilion), while in combination with pink it gives a true orange color.

According to STURTEVANT, pink is located close to Bare and the two gave only a few per cent of crossing over. An experiment involving the cross *bi p/Ba* ♀ with *bi p* ♂ gave 7.7 percent of crossing over between bithorax and pink and 4.2 per cent between pink and Bare (table 2). The sum of the two, 11.9, is somewhat higher than the value, 9.7 (table 4), that was obtained directly between bithorax and Bare. Despite the unexpectedly high number of observed double crossovers, it is justifiable to put the gene pink between bithorax and Bare.

Pauciseta[1] (*ps^1*) *and its allele pauciseta*[2] (*ps^2*). The gene pauciseta[1] (ps^1) was discovered by Miss GROSCURTH in the Chehalis-4 strain (race A), several generations after this strain had been derived from a single female caught in nature. The mutant is characterized by the absence of some bristles, especially the anterior dorso-centrals and the scutellars. In some cases, however, all bristles may be present, but one or both anterior dorso-centrals become somewhat more slender than normal. Unless the bristles are carefully examined, pauciseta[1] can be easily overlooked.

Pauciseta[2] was found by the author as a single female in progeny of a cross between $ct\ y\ m\ sn\ v\ se/s^2$ ♀ with $ct\ y\ m\ sn\ v\ se$♂ in March 1936. The fly had some bristles missing on both the thorax and the scutellum. It was mated with wild type males and produced exclusively wild type flies in F_1, which in F_2 produced 365 wild type and 122 pauciseta[2]. When pauciseta[2] males were mated to bithorax glass pauciseta[1] females, the progeny showed the pauciseta character, indicating that the new pauciseta is an allele of the old one. Since the two are indistinguishable, the new pauciseta may be designated as ps^2.

To localize the gene pauciseta in the second chromosome, two sets of experiments were carried out. They were $bi\ p\ ps/+$ ♀ by $bi\ ps\ ps$♂ and $bi\ gl\ ps/+$ ♀ by $bi\ gl\ ps$♂. The result as summarized in table 2 enables us to localize the gene pauciseta on the right end of the second chromosome.

The third chromosome

Orange (*or*). Orange eye color was found by LANCEFIELD and later described by CREW and LAMY (1934a). It is recessive and cannot be distinguished from the sex-linked vermilion eye color. The mutant can be easily classified even in combination with most other eye colors. The viability and productivity of the mutant fly are as good as in normal.

Purple (*pr*). The gene purple, another recessive eye color mutant, was found by CREW and LAMY (1932). It is a translucent color ranging from yellowish brown to chestnut. In males the testicular sheath appears colorless. When it is combined with orange, the eye becomes grayish white in color.

Scute (*Sc*). Scute, a dominant gene causing the absence of most bristles on the thorax and the head, was also reported by CREW and LAMY (1934a). The homozygous forms of Scute can be distinguished from the heterozygotes by the rough eyes and the absence of some microchaetae. Accord-

TABLE 3

Four point linkage experiments.

LOCI	TYPE OF CROSS	NON-CROSSOVERS		SINGLE C.O.						DOUBLE C.O.						TRIPLE C.O.		TOTAL
				REG 1		REG 2		REG 3		1, 2		1, 3		2, 3		1, 2, 3		
				Chromosome II														
Sm bi Ba gl	*bi gl*×*Sm Ba*	186	210	199	100	17	20	78	45	28	13	60	28	1	7	7	1	1000
				Chromosome III														
or ab Ja pr	*or ab pr*×*Ja*	286	146	14	43	44	24	108	45	4	8	4	4	6	2	1	2	741
or Ja Sc pr	*or Sc pr*×*Ja*	205	152	36	37	15	10	42	54	1	1	5	5	4	3	0	5	575
or Ja pr cv	*or pr cv*×*Ja*	273	202	39	40	85	98	59	68	12	7	17	4	7	8	2	1	922

ing to Crew and Lamy (1934a) Scute is located half way between orange and purple, each of which gives about 25 per cent of crossing over with Scute. Due to its favorable location and its clear cut expression, Scute has proved to be an extremely valuable mutant in this group.

Crossveinless (*cv*). The mutant crossveinless was reported by Crew and Lamy (1934b) as an autosomal recessive, which causes the absence of the posterior crossvein. The anterior crossvein may either be absent or incomplete. The mutant is easily distinguishable, if the wing is not damaged. According to Donald (1936) crossveinless is located 17 to 26 units from purple. This is in close agreement with my data, which give 18.0 per cent of crossing over between purple and crossveinless (table 4).

Table 4

Total data for each pair of loci.

CHROMOSOME II			CHROMOSOME III			CHROMOSOME IV		
LOCI	% OF CROSSING OVER	TOTAL FLIES EXAMINED	LOCI	% OF CROSSING OVER	TOTAL FLIES EXAMINED	LOCI	% OF CROSSING OVER	TOTAL FLIES EXAMINED
Sm bi	43.6	1000	*or Bl*	7.7	442	*in j*	17.1	1817
Sm p	41.2	542	*or ab*	9.7	1477	*in tg*	47.6	534
Sm Ba	22.8	1546	*or Ja*	15.8	2235	*in Cy*	50.1	468
Sm gl	46.5	1546	*or Sc*	21.8	1223	*j tg*	38.6	1055
Sm ps	50.0	542	*or pr*	36.7	3180	*j Cy*	41.9	1121
bi p	5.9	1126	*or cv*	42.5	922	*tg Cy*	11.5	987
bi Ba	9.7	1789	*Bl Sc*	17.0	442	*tg Ro*	10.5	354
bi gl	29.2	1250	*ab Ja*	12.3	741	*Cy Ro*	2.0	354
bi ps	41.6	895	*ab pr*	36.7	1477			
p Ba	4.2	481	*Ja Sc*	6.8	575			
p ps	39.6	1187	*Ja pr*	23.6	1497			
Ba gl	21.2	1546	*Ja cv*	38.0	922			
gl ps	18.0	250	*Sc pr*	21.6	781			
			Sc cv	45.6	655			
			pr cv	18.0	922			

Jagged (*Ja*). Jagged wing is a dominant mutation found by the author. Usually, only the inner margin of the wing is notched. In extreme cases, the whole wing may become strap-like and bear notches on all margins. Three such flies, two females and one male, appeared in a cross between ***bi gl/Sm Ba*** ♀ and ***bi gl*** ♂ in December 1935. A single Smoky Bare Jagged female was successfully crossed to three wild type males, producing 52 Jagged and 63 non-Jagged flies, equally distributed among both sexes. This shows that Jagged is an autosomal dominant mutation.

The test for the viability of the flies homozygous for Jagged wing indicates that Jagged is lethal when homozygous.

The locus for Jagged has been determined, by the combined results of several different type of four-point crossing over experiments (table 3), to lie 6.8 units to the left of the gene Scute (table 4).

Abrupt (*ab*). Abrupt longitudinal vein is a recessive mutant found by the author. It produces a shortening of the fourth longitudinal vein. In extreme cases, the vein may abruptly stop just below the posterior crossvein. The mutant cannot be distinguished from either short, a sex-linked gene causing the shortening of the third and fourth longitudinal veins, or short-4 which was described by CREW and LAMY (1935a) to produce the shortening of the fourth and fifth longitudinal veins. In order to avoid confusion, separate names are here proposed to designate these mutants. Short (*s*) remains to designate the sex-linked one. The fourth chromosome one, originally known as short-4 (s_4), is renamed incomplete (*in*). The name abrupt (*ab*) applies to the one in the third chromosome, which is now under discussion.

The origin of abrupt can be traced back to the orange purple stock. In three cultures of *na/or Sc pr* ♀ by *or pr* ♂, there appeared several males whose wings were indented at their inner margin. The gene concerned was found to be allelomorphic to the beaded originally described by LANCEFIELD (1922). It is known as bd^2. From a cross between *or* bd^2 and *or* sibs, three *or pr* females appeared to have their fourth longitudinal vein shortened. By mating them to wild type sibs, a good many orange abrupt purple flies of both sexes were obtained. Since all abrupt flies had orange purple eye color, the mutant must have originated in the *or pr* stock. When *or ab pr* flies were mated to incomplete (*in*), all F_1 individuals were wild type, indicating that abrupt and incomplete are not allelomorphs. An *or ab pr* stock was soon established. At the beginning some abrupt flies appeared to overlap normal. But after several generations of selection and inbreeding, the character became more pronounced and at the same time bred true.

The locus for the gene abrupt has been found to lie between orange and Jagged. As shown in table 4, it is closer to the former (9.7 units) than to the latter (12.3 units).

Blade (*Bl*). Blade wing is a dominant discovered by STURTEVANT (unpublished). The wing assumes a blade-like shape. It is easily classifiable and has normal viability and productivity. STURTEVANT crossed *or/Bl Sc* ♀ to *or* ♀ and found Blade to give 7.7 per cent of crossing over with orange and 17.0 per cent with Scute. With his permission the result of this cross is also included in table 2.

The fourth chromosome

Curly (*Cy*). Curly, a dominant gene, was discovered by Miss BEERS (1934) in race B. The wing may be curled either upward or downward.

Flies homozygous for Curly are always inviable. In heterozygous condition, the mutant fly is fully viable and fertile. Curly can be easily distinguished from normal. The gene has been successfully transferred from race B to race A.

Jaunty (*j*). The gene jaunty was reported by CREW and LAMY (1935a). It is recessive, and the mutant flies show a slight upturning of the tip of the wing. Occasionally, it overlaps normal.

Incomplete (*in*). The gene incomplete, another fourth chromosome recessive found by CREW and LAMY (1935a), was originally described by them as short-4. According to them incomplete and jaunty gave about 10 per cent of crossing over. Recently, DONALD (1936) reported that the two gave 15 to 16 per cent of recombinations. My data as shown in table 4, giving 17.1 per cent of crossing over, is in close agreement with the result of DONALD.

Multiple alleles of tangled (*tg*). The first mutant of tangled, tangled^1 (tg^1), was discovered by CREW and LAMY (1934b), who described it as fused. Later (1935a) they changed the name to tangled. It is recessive; in the mutant flies the second and third longitudinal veins come together at their distal ends, often with extra crossveins. The same also occurs with the fourth and fifth longitudinal veins. In extreme cases, the wing may be tilted up.

A single tangled^2 male, similar to tangled^1, except for having few extra veins, was found in Bare orange Curly stock. It was mated to wild type females, and in F_2 several *Ba or Cy* tg^2 and *or* tg^2 flies were obtained.

A single tangled^3 male fly found in the Curly stock had only one extra crossvein connecting the distal ends of the second and the third longitudinal veins. It was mated to wild type females, giving in F_1 all wild type flies and in F_2 8 tg^3 and 435 wild type ones. The great excess of wild type flies over the mutant type may be accounted for by presence of some modifier in the wild type parent, for after several generations of selection, tg^3 started to breed true.

A fourth allele of tangled, tg^4, was obtained by Dr. STURTEVANT in the echinus stock. The mutant flies resemble very much either tg^1 or tg^2. When they were crossed to each of the other three tangled alleles, all offspring were tangled.

Among the four alleles of tangled, tg^3 was most favorable for experimental purposes, because tg^3, having only one clear extra crossvein instead of the many characteristic of tg^1, tg^2, or tg^4, can be distinguished in combination with other fourth chromosome wing mutants. Moreover, tangled^3 wings, unlike other alleles of the same locus, are never tilted at the tip. Hence, for genetic analysis of the locus, tg^3 flies have been almost exclusively used.

According to DONALD (1936) tangled and jaunty gave 44 to 49 per cent of crossing over and the sequence of the genes was assumed to be incomplete-jaunty-tangled. My data, given in table 4, are in agreement with DONALD'S in regard to the sequence of genes but show less crossing over between *in* and *j*. This difference is probably due to the fact that the tangled used by DONALD was tg^1, which, being more extreme, may obscure the classification of flies in the combination of tangled and jaunty.

Rough (*Ro*). Rough is a dominant eye mutation found by Miss GROSCURTH in race A. It causes irregular arrangement of eye facets. Not infrequently the mutation overlaps normal. A single experiment involving the cross of tg^3 *Cy/Ro* ♀ by tg^3 ♂ gave 9.5 per cent of crossing over between tg^3 and *Cy* and 2.0 per cent between *Cy* and *Ro*. The absence of *Ro Cy* and tg^3 flies indicates that *Ro* is located to the right of *Cy*.

CONSTRUCTION OF THE GENETIC MAPS

According to MORGAN, STURTEVANT and BRIDGES (1925) four autosomal groups of genes were reported by LANCEFIELD with 4, 6, 1 and 2 recognized loci respectively. These, together with the sex-linked group, made the total number of linkage groups 5, which is equal to the haploid chromosome number of the species. In spite of the facts that the autosomal linkage groups reported by LANCEFIELD were imperfect, and that most of his mutants are now lost, some of them have been found to be the same as some of the ones described above. For this reason, the numbering of the second and the third linkage groups still follow the system of LANCEFIELD. The numbering of the fourth linkage group is justified on the basis of the cytological evidence which shows that the representative gene of this group is located in the rod-shaped chromosome and not in the chromosome now designated as the fifth, in which no gene is represented here.

A summary of linkage data is presented in tables 1–3. The first column at the left shows not only the loci concerned but also their sequence. Under the column headed "type of cross," types of two parents involved in the cross are shown, the one on the left being female and the one on the right male. In the third and the following columns classes are entered under the headings indicating the type of crossing over they represent. In every case the class which includes the individuals bearing the wild type allele of the most left-hand locus concerned is placed first, and is followed by the contrary class. The results are, of course, obtained from the backcrosses of the F_1 hybrids to multiple recessives.

Table 4 shows the total data for each pair of loci in the second, third and four chromosomes respectively. The resulting maps are shown in figure 1.

It is, of course, realized that these maps are only preliminary ones, and

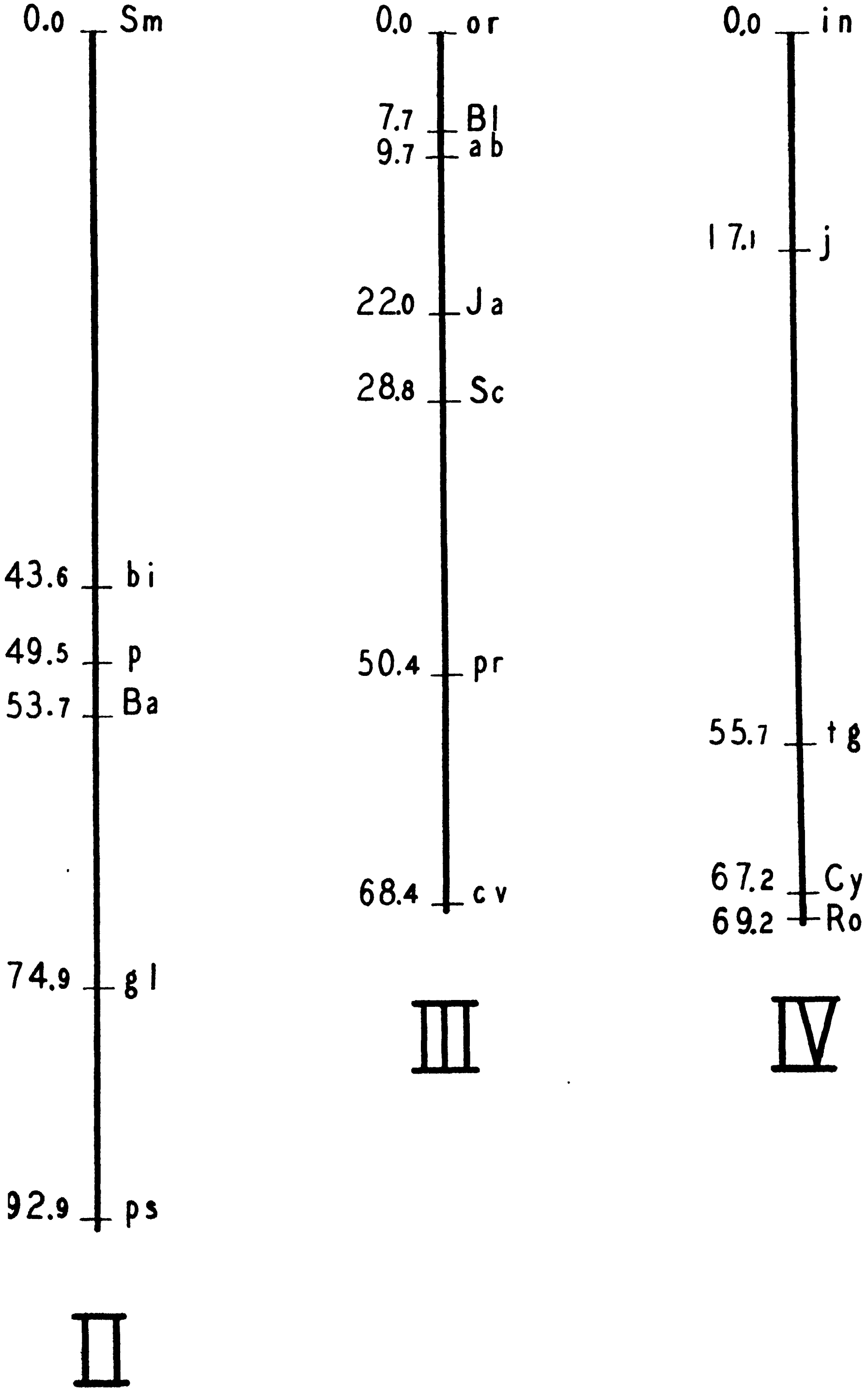

FIGURE 1.—Genetic maps of the three autosomes of *Drosophila pseudoobscura*. II—the second chromosome, III—the third chromosome, and IV—the fourth chromosome.

that both the total map-lengths and the distance between the genes may prove to be larger than here indicated. The data presented in table 4 are in general self consistent. Several apparent discrepancies may, however, be noticed; for instance, the value shown between Smoky and bithorax is slightly higher than between Smoky and pink or Smoky and Bare, although the maps show the reverse relation. Similar discrepancies are observed in the values between crossveinless and Scute and between crossveinless and Jagged. These discrepancies are probably due to differential viability of the classes concerned in different experiments.

In determining the map distances, the genes lying nearest to the spindle fibre attachment in each chromosome are taken as the zero points or the left ends. The evidence leading to the determination of the locations of the genes in relation to the spindle fibres will be published elsewhere in connection with the cytological maps. Suffice it to mention here that the loci known to lie nearest to the spindle fibre attachments are Smoky (*Sm*) in the second, orange (*or*) in the third, and incomplete (*in*) in the fourth chromosome. Hence, these loci are taken as zero points.

Recently, CREW and LAMY (1935a) attempted to homologize the second chromosome of *D. pseudoobscura* with the left arm of the V-shaped third chromosome of *D. melanogaster*, and the fourth chromosome of *pseudoobscura* to the right arm of the V-shaped second chromosome of *melanogaster*. In view of the facts that relatively few genes are now known in *D. pseudoobscura* and that the two species cannot be crossed to effect a direct test, it seems to the author that attempts to establish the homology of genetic maps of the two species are rather hazardous. Indeed, DOBZHANKSY and TAN (1936) show that even the two closely related species, *D. pseudoobscura* and *D. miranda*, have no single chromosome in common. *D. pseudoobscura* and *D. melanogaster*, being much less closely related, have the gene arrangement in their chromosomes altered beyond recognition.

The author wishes to express his gratitude to Professors TH. DOBZHANSKY and A. H. STURTEVANT for encouragement, advice and use of their stocks.

SUMMARY

1. The mutants belonging to the three autosomal linkage groups of *Drosophila pseudoobscura* are briefly described.

2. The genetic maps of the three autosomes of the species are constructed as shown in figure 1.

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PHENOGENETIC STUDIES IN SCUTE OF D. MELANOGASTER
III. THE EFFECT OF TEMPERATURE ON SCUTE 5

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INTRODUCTION

THIS paper deals with the quantitative variations, at different temperatures, in the bristle numbers of *sc 1*, *sc* 5, and their compound *sc 1/sc 5*. The allele *sc 5* was found by GAISSINOVITSCH (1930). According to DUBININ (1929, 1932), *sc 5* is a less extreme allele than *sc 1*. (1) *Sc 5* effects fewer bristles and has a lesser effect on those bristles. (2) The bristles affected by both *sc 1* and *sc 5* are the only ones affected in the compound *sc 1/sc 5*. (3) The mean bristle numbers of the compound are intermediate between *sc 1* and *sc 5*.

It will be shown in this paper that these generalizations do not hold at all temperatures for all the bristles. These experiments further add to the growing amount of quantitative data which ultimately must be used to formulate definitely and test a quantitative theory of development such as proposed by GOLDSCHMIDT (1927) and PLUNKETT (1926).

MATERIALS AND METHODS

Culture conditions

The usual Drosophila culture methods were employed (banana-agar gel with added yeast) with added precautions to insure uniform conditions for large numbers of flies raised in half-pint bottles (CHILD 1935). To insure that the *sc 5*, *sc 1*, and the *sc 1/sc 5* flies had as similar an environment as possible, they were raised in the same culture in most of the experiments. Two methods were used to obtain the various genotypes in the same bottles. In some experiments males and females of both alleles were mated in the same bottle. In other experiments *sc 1/sc 5* flies were mated to *sc 1* and *sc 5* males in the same bottle.

Flies 4–7 days old raised under favorable conditions were used for egg laying. About 20–30 females were used per bottle with an egg-laying period of from 1 to 3 hours duration. The temperature was regulated in incubators held constant to within 0.1°.

Selection

The details of selection of the *sc 1* stock and the tests for the adequacy of selection were presented in a previous communication (CHILD 1935).

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* This investigation was aided by a grant from the Rockefeller Foundation.

The *sc 5* flies were made isogenic with the *sc 1* by twenty generations of backcrossing *sc 1/sc 5* by *sc 1* males. The only difference between these *sc* stocks is, therefore, the possession of one or the other of the *sc* alleles.

The original *sc 5* stock was actually *y sc 5*. During the course of these experiments one crossover was obtained between *y* and *sc 5*, thus eliminating the yellow. The bristle numbers of *y sc 5* and *sc 5* were found to be the same, however, and in reporting these data no distinction will be made as to whether the flies were yellow or wild type for color.

EXPERIMENTAL DATA

The flies were raised for their total period of development at temperatures ranging from 14° to 30°. The complete and more extensive data relating to the effect of temperature on the mean bristle numbers of *sc 1* have been reported previously (CHILD 1935). The *sc 1* flies in these experiments served, therefore, as controls. Those few experiments were discarded in which the bristle numbers of the *sc 1* flies differed from the results previously obtained. This type of control served as a very careful check on the temperature and the culture conditions.

TABLE 1*

Effect of Temperature on mean bristle numbers.

Males

T±0.1°	*M a sc*		*M p sc*		*M i v*	
	sc 5	*sc 1*	*sc 5*	*sc 1*	*sc 5*	*sc 1*
14°	.11±.019	.020±.014	.24±.027	.060±.024	.62±.030	.68±.046
15°	.11±.023	.019±.004	.26±.032	.035±.005	.72±.033	.94±.029
16°	.10±.015	.027±.012	.14±.018	.049±.016	.91±.015	.99±.005
18°	.09±.018	.004±.002	.57±.014	.005±.002	.99±.006	1.
19°	.13±.036	.012±.012	.69±.015	.002±.001	1.0	1.
20°	.18±.036	.005±.001	.86±.026	.0	1.	1.
22°	.19±.033	.005±.003	.69±.021	.0	1.	1.
23°	.21±.017	.025±.014	.09±.004	.0	1.	1.
24°	.35±.036	.020±.014	.11±.005	.0	1.	1.
25°	.22±.022	.003±.002	.00	.0	1.	1.
26°	.28±.025	.008±.008	.06±.004	.0	1.	1.
27°	.27±.024	.007±.003	.00	.0	1.	1.
28°	.28±.025	.004±.002	.06±.004	.0	1.	1.
29°	.32±.023	.005±.002	.05±.004	.0	1.	1.
30°	.17±.016	.000	.22±.007	.0	1.	1.

*** a sc, p sc, anterior and posterior scutellars; iv, pv, inner and posterior verticals; oc, ocellar.**

The results of these experiments are presented in tables 1 and 2, and are illustrated in figures 1 and 2. At each temperature, between 50 and 300 flies of each type were counted. The results are given as mean bristle

TABLE 2

Effect of temperature on mean bristle numbers.

Females

T ± .1°	M p sc			M pv		
	sc 5/sc 5	sc 1/sc 5	sc 1/sc 1	sc 5/sc 5	sc 1/sc 5	sc 1/sc 1
14°	.54 ± .054	.47 ± .033	.28 ± .033	1.	.93 ± .017	.020 ± .019
15°	.54 ± .048	.42 ± .049	.23 ± .012	1.	.98 ± .014	.060 ± .006
16°	.46 ± .035	.35 ± .028	.15 ± .025	1.	.97 ± .010	.045 ± .015
18°	.36 ± .029	.20 ± .028	.07 ± .077	1.	.95 ± .015	.077 ± .088
19°	.34 ± .034	.18 ± .041	.04 ± .025	1.	.95 ± .023	.036 ± .025
20°	.25 ± .036	—	.02 ± .001	1.	—	.086 ± .008
22°	.09 ± .024	.09 ± .031	.01 ± .004	1.	.97 ± .018	.045 ± .009
23°	.07 ± .015	.08 ± .019	.01 ± .002	1.	.98 ± .009	.028 ± .014
24°	.09 ± .023	.04 ± .016	.00	1.	.98 ± .009	.000
25°	.06 ± .018	.04 ± .020	.01 ± .009	1.	.96 ± .020	.012 ± .003
26°	.04 ± .015	.02 ± .011	.00	1.	.94 ± .018	.009 ± .009
27°	.05 ± .017	.00	.00	1.	.97 ± .013	.000
28°	.06 ± .016	.01 ± .009	.00	1.	.96 ± .018	.001 ± .001
29°	.05 ± .012	.03 ± .012	.00	1.	.78 ± 031	.000
30°	.06 ± .015	.03 ± .011	.00	1.	.74 ± .026	.000

T ± .1°	M oc			M a sc		
	sc 5/sc 5	sc 1/sc 5	sc 1/sc 1	sc 5/sc 5	sc 1/sc 5	sc 1/sc 1
14°	1.	.93 ± .017	.00	.14 ± .037	.18 ± .025	.04 ± .026
15°	1.	.94 ± .024	.00	.13 ± .035	.18 ± .038	.09 ± .008
16°	1.	.96 ± .011	.00	.20 ± .028	.12 ± .019	.08 ± .021
18°	1.	.98 ± .009	.00	.25 ± .027	.23 ± .028	.09 ± .009
19°	1.	.99 ± .011	.00	.38 ± .035	.39 ± .052	.07 ± .035
20°	1.	—	.00	.49 ± .042	—	.13 ± .003
22°	1.	.99 ± .011	.00	.61 ± .039	.50 ± .054	.17 ± .006
23°	1.	1.	.01 ± .007	.63 ± .028	.55 ± .034	.22 ± .031
24°	1.	1.	.04 ± .018	.68 ± .037	.58 ± .033	.24 ± .040
25°	1.	1.	.08 ± .008	.69 ± .033	.51 ± .050	.17 ± .011
26°	1.	1.	.40 ± .050	.64 ± .036	.50 ± .038	.12 ± .033
27°	1.	1.	.41 ± .021	.61 ± .038	.46 ± .039	.14 ± .015
28°	1.	1.	.60 ± .013	.62 ± .033	.50 ± .044	.06 ± .006
29°	1.	1.	.75 ± .017	.61 ± .029	.50 ± .037	.05 ± .009
30°	1.	1.	.85 ± .020	.52 ± .030	.45 ± .030	.05 ± .009

T ± .1°	M i v		
	sc 5/sc 5	sc 1/sc 5	sc 1/sc 1
14°	.94 ± .022	.93 ± .018	.98 ± .019
15°	.99 ± .01	1.	1.
16°	1.	1.	1.

M. i.v. = 1 at higher temperatures.

numbers per half fly. The *sc 1* curves contain additional points from data previously obtained (CHILD 1935).

DISCUSSION OF RESULTS

With one exception, the i.v.,[1] the bristle numbers of the *sc 5* stock are higher than the *sc 1*. With respect to the i.v. bristle, *sc 5* is therefore a more extreme allele than *sc 1* (fig. 2). Furthermore, as pointed out previously (CHILD 1935) this variation in the M. i.v. is contrary to the subgene hypothesis (DUBININ 1929, 1932, GAISSINOVITSCH 1930), since the i.v. is in a center outside both the *sc 1* and *sc 5* centers as postulated by the Russian workers.

It is further evident from these data that the mean bristle number of a particular bristle (if at all variable) varies in the same way with temperature in the *sc 1*, *sc 5*, and *sc 1/sc 5* stocks.

The bristle numbers of the *sc 1/sc 5* are intermediate between *sc 1* and *sc 5*, although this relationship is somewhat obscured at low temperatures for the asc. The mean bristle numbers of *sc 1/sc 5*, moreover, are closer to those of *sc 5* than *sc 1*. In other words, *sc 5* appears to be "dominant" over *sc 1*. However, the degree of this dominance is not the same for all bristles, and, furthermore, it is not the same for the same bristle at different temperatures.

The data on the pv and the oc bristles (these should not have varied in *sc 1/sc 5* according to subgene hypothesis) illustrate a problem which has been recognized by Drosophila workers on other characters; PLUNKETT (1926) on Dichaete, MOHR (1932) on vestigial, BARON (1935) on eyeless, TIMOFEEF-RESSOVSKY (1934) on venation, and others. This point can be best made clear by citing specific examples. MOHR (1932) found that vg^{ni}/vg^{ni} (nicked) and the wild type are alike phenotypically, both giving normal wings. This, however, does not imply that the concentration of some substance or substances producing the character, normal wings, is the same in both genotypes. This was shown by comparing nicked and the normal allele in combination with the other mutant alleles of vestigial. In combination with *vg* alleles, the character nicked could be discerned. Other slight *vg* alleles showed a similar behavior, their expression depending also upon environmental conditions, especially temperature. BARON (1935) in analyzing isogenic populations of *ey* (eyeless) drosophilae found that there was a variation in the amount of eye producing substance among the "no-eyed" eyeless, although all the "no-eyed" flies had less than the amount needed to produce eyes.

At Minnesota with Dr. HUTT (data unpublished) I found a similar situation in a character in Gallus known as "down defect," a bald spot

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[1] For meanings of the bristle abbreviations see footnote to table 1.

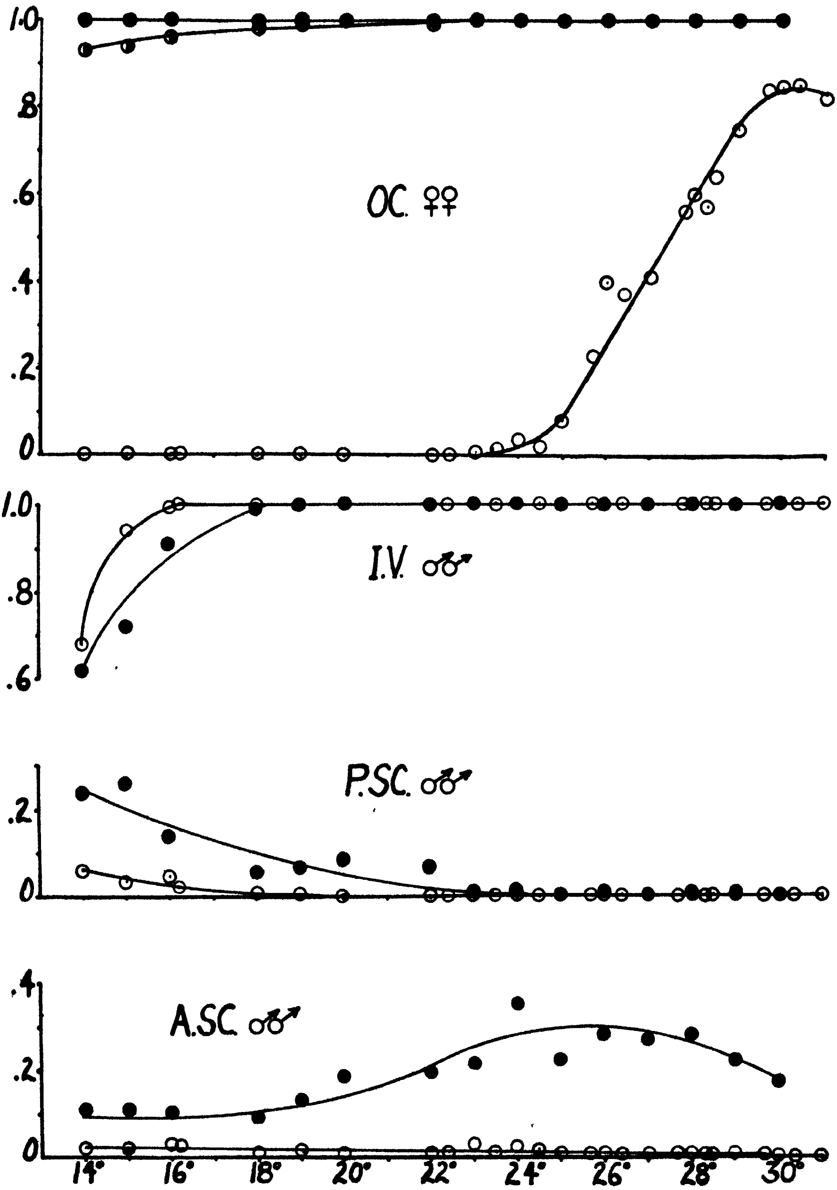

FIGURE 1.—Effect of temperature on mean bristle number. Ordinates, mean bristle numbers; abscissae, temperature in °C.

○ = *sc* 1   ⊙ = $\frac{sc\ 1}{sc\ 5}$ ● = *sc* 5

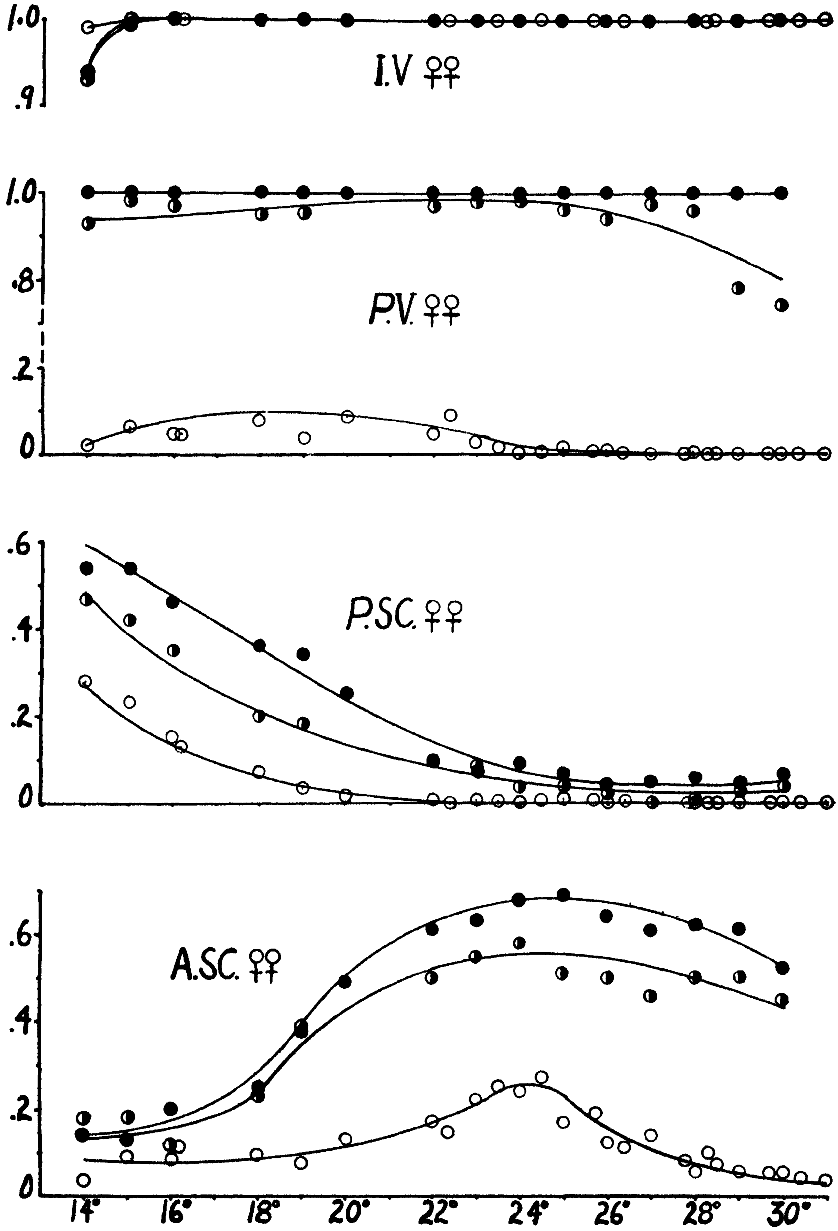

FIGURE 2.—Effect of temperature on mean bristle number. Ordinates, mean bristle number; abscissae, temperature in °C.

○ = *sc* 1   ◑ = $\frac{sc\ 1}{sc\ 5}$ ● = *sc* 5 on the head of the fowl. In populations homozygous for down defect there is a variation in the size of the bald spot, a certain percentage of the fowls not showing the character at all, although genetically down defect. A statistical analysis of such a population indicated that a definite percentage of "normal" fowl were to be expected, if one assumed a distribution in amount of "down defect producing substance" among the individuals of a homozygous down defect population. The "normal" fowl in this analysis had varying quantities of a smaller amount of "down defect producing substance" than the threshold amount needed to show the character.

In all these instances where the expression of a character depends upon a threshold amount of character producing substance, any amount less than the threshold will have no effect. An analogous situation may be seen in muscle contraction where any subminimal stimulus evokes no response. If the character is variable such as wing or eye size, varying amounts of substance above the threshold may produce varying degrees of character expression. With respect to bristle production, however, the situation is more complex. A particular bristle is either present or absent, like the all-or-nothing principle in the contraction of a single muscle fiber. Any amount of substance above the threshold amount will produce only one bristle.

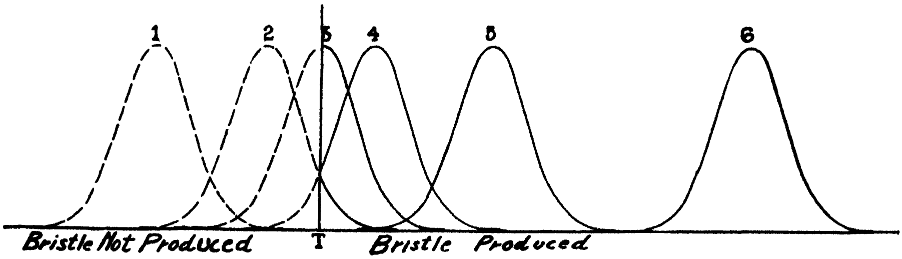

FIGURE 3.—Hypothetical distributions in concentrations of bristle producing substance for a particular bristle. The numbers 1–6 refer to various populations. T is the threshold concentration.

The effect of the threshold with respect to a bristle is illustrated in figure 3. Populations such as 2–4 will have mean bristle numbers between 0 and 1 (curve 2 near 0, curve 4 near 1). Populations which do not cross the threshold value will have mean bristle numbers of 0 if on the left or of 1 if on the right of threshold, although these populations differ from one another, for example 5 and 6.

In *sc 5* (fig. 2) the oc bristle is never affected (is like the wild type) at all temperatures (like curves 5, 6 in fig. 3). In *sc 1* the oc bristle is absent in all flies raised under 22° (like curve 1 in fig. 3). The M. oc increases rather sharply with increasing temperature (like curves 2–4). In *sc 1/sc 5* the M. oc equals 1 above 20°, and is less than 1 below 20°. These results indicate that although the M. oc in *sc 5* is 1 at all temperatures, the concentration of bristle producing substance is not the same at all temperatures and similarly for *sc 1* below 22°. The p.v. bristle (fig. 2) behaves in a somewhat similar manner. The temperature effect on *sc 1/sc 5* indicating that either *sc 1/sc 1* and *sc 5/sc 5* or both have a decreasing amount of bristle producing substance with increasing temperature above 25°. In *sc 1 sc 1*, since the amount is already below the threshold (curve 1 and 2, figure 3), no further effect on the bristle number can be seen. In *sc 5/sc 5* the inference is that the amount of bristle producing substance although decreasing above 25° does not cross the threshold.

According to this interpretation, in order to use mean bristle number as an exact quantitative character in a study of the chemical kinetics involved in bristle production, a statistical or experimental analysis of one bristle and no bristle must be made. PLUNKETT (1926) found that because of the arbitrary assumptions needed in a statistical analysis, a satisfactory result could not be obtained. It is hoped that experimental studies with combinations of other scute alleles will shed more light on the problem.

A study of the temperature effective periods of these stocks is being made. Preliminary results indicate that both the temperature-effective periods and the time of development differ in these three stocks.

SUMMARY

1. The mean bristle numbers in isogenic stocks of *sc 1*, *sc 5*, and *sc 1/sc 5* were determined at temperatures ranging from 14° to 30°.

2. *Sc 1* is a more extreme allele than *sc 5* for all bristles affected except the i.v. bristle.

3. The effect of temperature on the mean bristle number is in the same direction in the three stocks.

4. The mean bristle number of *sc 1/sc 5* is intermediate between *sc 1* and *sc 5* at all temperatures.

5. The problem of thresholds as applied to the presence and absence of a particular bristle is discussed.

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STUDIES ON THE BAR SERIES OF DROSOPHILA
III. THE FACET RELATIONS IN DORSAL AND VENTRAL LOBES OF THE EYE

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INTRODUCTION

THE relative growth of parts of many organisms has been shown to conform to the simple power function

$$y = bx^k$$

where y and x represent the parts measured, and b and k are constants. When y and x are plotted on a logarithmic scale a straight line is obtained. The many applications of this equation to growth data have been fully reviewed by HUXLEY (1932). More recently, HERSH (1934) and ROBB (1935a, 1935b) have extended the application of the relative growth function from the purely ontogenetic sphere to the phylogenetic, through investigations on the evolutionary relative growth of parts in the Titanotheres and the horse, respectively.

The first attempt to apply the relative growth function to data on the relative sizes of parts in Drosophila was made by HERSH (1928). The well known lobing of the compound eye in Bar-eyed flies makes possible a quantitative determination of the relative sizes of the two lobes expressed in terms of facet number. In a series of investigations on Bar raised at different temperatures (HERSH 1928) and on different Bar stocks raised at the same temperature (HERSH 1931), HERSH concludes that the relative growth function adequately expresses the observed relations between facet number in the dorsal and ventral lobes of the eye.

In my own investigations on facet number in Bar (MARGOLIS 1935), separate records of facet number in the two lobes of the eye were kept in so far as possible. These data, together with others gathered subsequently onan unrelated Bar stock obtained from DR. A. L. BARON, serve as the material for the present analysis of the facet relations in dorsal and ventral lobes of the eye. My own results differ from those of HERSH in several respects, particularly with reference to the applicability of the relative growth function. At the same time, the data suggest a possible basis for the observed differences and are accordingly presented.

EXPERIMENTAL

Stocks

The data on facet number are taken from two unrelated but genetically homogeneous Bar stocks. The stock designated as A is the one used in my earlier experiments on the effect of the gene vestigial on facet number in Bar. The details concerning inbreeding, genetic homogeneity, culture methods, facet number for the whole eye, etc., have been presented elsewhere (Margolis 1935). The stock designated as B is the one obtained from Dr. A. L. Baron. This stock had previously been rendered isogenic with a wild Oregon stock used by Dr. Fowsner in his experiments on the duration of various developmental periods at different temperatures (Powsner 1935). The general method of rendering the two stocks isogenic through the use of chromosomes with dominant markers and crossover suppressors has already been outlined (Baron 1935). Before using stock B in my own experiments, the stock was inbred with a vestigial stock by pair brother-sister matings for fifteen generations. This vestigial stock had previously been backcrossed to Powsner's wild stock for 34 generations. This latter procedure was carried out in order to continue my earlier experiments on Bar and vestigial. The method insures a high degree of genetic uniformity in both the Bar and vestigial stocks and, at the same time, a close relationship to the wild stock of Powsner. Since this latter stock has shown such excellent viability, fertility, and genetic homogeneity, a number of workers in our laboratory are using it as a standard wild-type with which the developmental effects of various mutant characters may be compared. The actual degree of genetic uniformity obtained in stock B by the above procedure, as measured by the parent-offspring correlation, will be discussed later.

Methods

The experimental methods employed in securing the data for stock A have been described elsewhere (Margolis 1935). A series of seven experiments were conducted using several temperatures in each experiment, and extending over a period of a year and a half.

The data on facet counts in stock B were taken from a series of experiments dealing with a preliminary localization of the temperature-effective period for facet determination in this stock, and from other experiments dealing with the effect of reduced atmospheric pressures on facet number. None of the data on stock B have been published previously and are presented here strictly from the point of view of determining the relation between dorsal and ventral lobes of the eye in respect to facet number. The experiments on the effect of reduced atmospheric pressures proved negative and will be considered elsewhere as one aspect of the effects of various environmental agencies on facet number in Bar. The facts relating to culture methods, temperature control, and general uniformity of environmental factors are the same for stock B as for stock A.

Facet counts

In making the facet counts on both stocks, separate records of facet number in the two lobes were kept where possible. In the case of Bar there is always some indication of lobing of the eye; but in the larger eyes, especially at low temperatures, there is a tendency for the two lobes to merge, making accurate discrimination between lobes difficult. I found it extremely difficult to distinguish accurately between lobes at temperatures below 22°C, so that the data below this temperature are far too meager for analysis and are not given. At all temperatures, personal error in distinguishing between lobes was minimized by recording the lobes separately only in cases where clear definition was possible. A comparison of the total eye size of flies in which dorsal and ventral lobes were clearly distinguishable with the total eye size of the population as a whole, at each temperature, indicates that the former is truly a random sample of the latter. This fact is easily verified by comparing table 1 of this paper with table 3 in the paper previously cited (MARGOLIS 1935).

Data on facet number in dorsal and ventral lobes

Throughout this paper the mean facet number in the dorsal lobe at any temperature will be designated as D, and correspondingly the ventral lobe facet number as V. In table 1 are given the values of D and V, respectively, for different temperatures, together with their standard deviations. The column headed D/V gives the ratio of D to V and the column headed r, the correlation between lobes.

A number of interesting facts are apparent in the data. In those cases where experiments at a given temperature are repeated one or more times the values of D and V, in general, are similar within the limits of sampling error. The ratio, D/V, differs for the two sexes scattering narrowly around a value of 1.15 for the males and 1.0 for the females. For either sex the ratio is relatively constant over the temperature range. The values of the correlation coefficients (r) present the most interesting feature of the table. There is, in general, a fairly systematic decrease in the value of r with increase in temperature. At the same time, however, where experiments at a given temperature are duplicated there are in some cases significant differences in the values of r for any one temperature. This is especially noticeable in the case of the four experiments at 28°. This temperature was used as a control temperature throughout the series of experiments, and although the mean facet numbers display an insignificant scattering in the different experiments there are significant differences in r. This indicates an inadequate control of some factor or factors which influence variation in facet number in the two lobes, but which do not perceptibly shift the

TABLE 1

Statistics on facet number in dorsal and ventral lobes of stock A.

EXP.	SEX	T	D	σ_D	V	σ_V	D/V	r	n
IV	♂	22°	57.9±1.7	7.90	50.0±1.5	6.85	1.16	0.600±0.136	22
	♀	22°	51.2±1.3	6.60	51.6±1.3	6.60	0.99	0.279±0.181	26
V	♂	22°	58.6±2.3	9.20	52.0±2.0	8.10	1.13	0.902±0.047	16
	♀	22°	52.9±1.3	5.05	53.6±1.2	4.90	0.97	0.881±0.056	16
VII	♂	22°	58.2±2.1	8.85	48.0±2.1	8.85	1.21	0.690±0.124	18
	♀	22°	50.3±1.5	7.20	52.6±1.6	7.89	0.96	0.701±0.104	24
VII	♂	24°	45.4±1.3	7.26	37.9±1.1	6.39	1.20	0.515±0.129	32
	♀	24°	40.1±0.8	5.76	40.4±0.9	6.45	0.99	0.612±0.089	50
I	♂	25°	40.7±1.1	4.88	34.4±1.0	4.62	1.18	0.677±0.120	20
	♀	25°	38.6±1.0	5.34	35.7±0.8	4.29	1.08	0.555±0.131	28
VII	♂	25.8°	36.9±0.8	5.40	31.6±0.8	5.25	1.17	0.556±0.105	43
	♀	25.8°	35.5±1.1	5.31	34.7±1.2	5.49	1.02	0.680±0.114	22
II	♂	27°	31.3±0.8	4.40	27.2±0.6	3.52	1.15	0.448±0.145	30
	♀	27°	28.7±0.7	3.90	29.9±0.8	4.82	0.96	0.628±0.106	33
III	♂	27°	31.3±0.4	4.00	27.9±0.4	4.20	1.12	0.427±0.077	112
	♀	27°	28.6±0.4	3.92	29.1±0.5	4.26	0.98	0.398±0.090	88
II	♂	28°	27.3±0.6	4.18	24.3±0.5	3.54	1.12	0.634±0.081	53
	♀	28°	22.4±0.7	4.10	23.3±0.6	3.74	0.96	0.147±0.157	39
III	♂	28°	26.3±0.7	4.24	22.8±0.5	2.96	1.15	0.488±0.121	40
	♀	28°	26.4±0.7	4.08	22.3±0.6	3.40	1.18	0.404±0.146	33
IV	♂	28°	25.7±0.6	3.60	23.6±0.6	3.18	1.09	0.382±0.149	33
	♀	28°	25.4±0.8	4.48	25.0±0.7	3.68	1.02	0.224±0.173	30
VI	♂	28°	27.1±0.7	4.14	25.3±0.4	2.52	1.07	0.161±0.170	33
	♀	28°	23.7±0.8	3.78	25.8±0.2	3.66	0.92	0.164±0.203	23
I	♀	29°	22.6±0.8	3.24	19.8±0.6	2.44	1.14	0.063±0.249	16
III	♂	29°	22.0±0.5	3.40	20.2±0.4	2.46	1.09	−0.092±0.143	48
	♀	29°	20.9±0.5	3.06	19.0±0.4	2.38	1.10	−0.076±0.166	36
II	♂	30°	22.0±0.7	2.58	20.1±0.5	1.85	1.09	0.522±0.195	14
	♀	30°	19.8±0.6	2.24	18.7±0.4	1.68	1.06	0.017±0.250	16
III	♂	30.4°	17.3±0.5	2.35	14.8±0.6	2.87	1.17	0.225±0.194	24
	♀	30.4°	17.0±0.3	1.93	15.9±0.4	2.34	1.07	0.356±0.152	33
I	♀	31°	17.6±0.3	1.03	15.8±0.4	1.73	1.11	−0.231±0.231	17

mean value for the population. As will be seen later, this feature in the data appears also in stock B. There is, as one should expect, a close approximation in the values of r for the two sexes. The few exceptions to this fact will be considered in connection with the data in table 2.

The biometric constants for facet number on stock B are contained in

TABLE 2

Statistics on facet number in dorsal and ventral lobes of stock B.

EXP.	SEX	T	D	σ_D	V	σ_V	D/V	r	n
IIb	♂	22°	69.1±1.1	6.49	42.2±0.8	4.71	1.64	0.535±0.121	35
	♀	22°	48.3±0.9	5.85	42.9±0.8	4.91	1.13	0.252±0.146	41
IIc	♂	22°	79.7±1.3	6.45	40.6±0.9	4.50	1.61	−0.237±0.193	24
	♀	22°	49.0±0.9	4.08	44.1±1.3	5.81	1.11	0.332±0.199	20
IId	♂	22°	77.6±1.6	6.30	50.2±1.6	6.05	1.55	0.188±0.250	15
	♀	22°	47.8±0.8	4.56	43.2±1.2	6.77	1.11	0.221±0.165	33
IIa	♂	28°	43.4±0.6	5.72	26.2±0.3	3.31	1.66	0.192±0.092	110
	♀	28°	27.7±0.3	3.36	22.8±0.3	2.90	1.22	0.455±0.072	122
IIIa	♂	28°	44.4±0.8	6.38	27.8±0.5	3.80	1.60	0.391±0.106	64
	♀	28°	28.5±0.5	3.01	23.8±0.5	3.01	1.20	−0.190±0.145	44
IIIb	♂	28°	41.4±0.6	4.50	26.1±0.3	2.40	1.59	0.200±0.133	52
	♀	28°	29.6±0.6	3.79	23.2±0.5	3.08	1.28	0.250±0.154	37
IIIc	♂	28°	42.4±0.8	5.74	26.4±0.4	2.67	1.61	0.190±0.130	55
	♀	28°	29.9±0.5	3.08	23.0±0.5	3.17	1.30	0.251±0.159	35
IIId	♂	28°	41.2±0.9	5.87	25.3±0.4	2.70	1.63	0.156±0.143	46
	♀	28°	30.3±0.6	3.12	22.4±0.5	2.74	1.35	0.252±0.184	26
IIIe	♂	28°	45.2±1.0	6.92	26.5±0.5	3.48	1.71	0.350±0.125	49
	♀	28°	30.4±0.7	4.02	23.3±0.5	2.87	1.30	−0.048±0.166	36
IIIf	♂	28°	43.6±1.0	5.54	25.9±0.6	2.98	1.68	0.082±0.187	28
	♀	28°	30.6±0.8	3.88	23.0±0.5	2.63	1.33	0.141±0.192	26
IIIg	♂	28°	43.4±0.9	6.10	26.7±0.5	3.20	1.63	0.512±0.104	50
	♀	28°	32.8±0.7	3.72	25.0±0.8	4.52	1.31	0.276±0.168	30
IIIh	♂	28°	41.0±0.8	5.14	24.7±0.4	2.58	1.66	0.114±0.147	45
	♀	28°	30.8±0.6	3.70	22.5±0.5	3.18	1.37	−0.189±0.163	35
IIIi	♂	28°	46.0±0.9	4.26	25.6±0.7	3.14	1.80	0.107±0.206	23
	♀	28°	31.6±0.6	2.50	22.8±0.5	2.20	1.39	0.115±0.233	18
Ia	♂	28°	42.7±0.7	5.71	27.6±0.5	3.54	1.55	0.314±0.108	58
	♀	28°	29.0±0.6	3.00	24.0±0.6	2.90	1.21	0.188±0.182	28

table 2, and are arranged in the same manner as table 1. Only two temperatures are represented in the various experiments. Although data at other temperatures are desirable, these data in themselves clearly bring out certain features of the facet relations in dorsal and ventral lobes. In so far as duplication of means is concerned the experiments are entirely satisfactory with the exception of experiment IIb at 22°. The scattering in values of D and V at 28° does not appear to be significant statistically although there is a slight trend when the male and female values for different experiments are compared. This indicates some slight variation in experimental conditions from one experiment to another, too small to be detected in the sampling errors of the means in populations of this size.

The values of D/V differ for the sexes, although they appear to be relatively constant for the two temperatures in the males. There is some indication that D/V is lower at 22° than at 28° for the females. The correlation between lobes is, in general, small and in most individual experiments not statistically significant. Viewing the data as a whole there is no doubt that a small but significant positive correlation between lobes exists. The basis for this correlation may rest upon some residual genetic variability, upon some inadequately controlled environmental variable, or finally upon some sort of developmental interdependence between the two lobes. If development of facets is independent in the two lobes, then in a genetically homogeneous stock raised under uniform environmental conditions the correlation between lobes should be 0. In this event, the presence or absence of a significant correlation may be used as a test for the uniformity of all factors which determine facet number in any experiment.

An experiment was designed to test the degree to which genetic heterogeneity in stock B might account for the observed correlation between lobes. A parent-offspring correlation for facet number was carried out at 28°. In both the parents and the offspring separate records of dorsal and ventral lobes in left and right eyes were kept. Table 3 contains the data on the parent-offspring, left-right, and dorso-ventral correlations in stock B. The slight discrepancy in numbers of individuals on which each correlation coefficient is based is due to the previously mentioned fact that clear discrimination of lobes is not always possible, coupled with the fact that in some cases one or the other eye cannot be counted due to mechanical injuries of one sort or another.

The values of r for parent-offspring and for dorso-ventral lobes are very similar in both males and females. The small differences which appear are probably sampling errors. The left-right correlations, on the other hand, are much larger and differ significantly from the other correlation coefficients. This latter fact confirms my view that in Bar some form of developmental dependence exists between the two eyes (MARGOLIS 1935).

Since the parent-offspring correlation serves as a quantitative measure of the degree of genetic heterogeneity in a population, one may conclude that in this experiment genetic heterogeneity, in itself, is sufficient to account for the observed correlation between dorsal and ventral lobes. Environmental variations, if present, will increase the correlation. The same is true for any sort of developmental interdependence, as demonstrated by the left-right correlations.

The fact that residual genetic heterogeneity adequately accounts for the dorso-ventral correlation is further supported by the values of r at 28° in table 2. The great majority of these values in both sexes closely approximate the values of the parent-offspring correlations in table 3. The few exceptions which show high positive correlation may be due to some inadequately controlled environmental variable which escaped attention during the experiments.

TABLE 3

Parent-offspring, left-right, and dorso-ventral correlations in stock B taken from the same set of data.

MALES

r(parent-offspring) = 0.188 ± 0.060; n = 256
r(left-right) = 0.483 ± 0.050; n = 235
r(dorso-ventral) = 0.273 ± 0.061; n = 228

FEMALES

r(parent-offspring) = 0.246 ± 0.069; n = 182
r(left-right) = 0.560 ± 0.056; n = 150
r(dorso-ventral) = 0.196 ± 0.074; n = 170

Attention has already been directed to those cases in table 1 where a significant difference in r between males and females is observed. A few similar cases are present in table 2. It is perhaps noteworthy that, with a single exception (table 2, exp. IIa), wherever a large difference in r exists, the males show the higher value. Formally, at least, this fact can be explained by assuming the presence of sex-linked recessive modifiers of facet number which are not uniformly distributed in the population. The presence of sex-linked modifiers of Bar is known from the experiments of HERSH (1929). Such modifiers would affect the facet number in the males, but might be masked in the females through the effects of more uniformly distributed dominant alleles. An alternative explanation in terms of differential effects of environmental variables on the two sexes is tenable but appears less probable.

The systematic trend in r for stock A (table 1) is interesting in the light of the explanation offered here for the correlation observed between lobes. One should expect to find the values of r scattering about some central value representative of the parent-offspring correlation, if the interpretation given for stock B applies equally to stock A. The parent-offspring correlation for stock A at 28° was found to be 0.17 ±0.067 for the males, and 0.25 ±0.065 for the females. At 28°, with the exception of males in experiment II, the values of r do not depart widely from the values for the parent-offspring correlation. It is obvious that at all temperatures below 28° the values of r are much higher than one is led to expect from the parent-offspring correlation. This raises an important question as to whether a routine determination of a parent-offspring correlation with reference to any quantitative character under a single set of conditions, for example a single temperature, is sufficient for establishing the degree of uniformity of genetic factors affecting that character. From our knowledge of the effects of environmental agencies on development in general, it appears probable that parent-offspring correlations will differ under different sets of conditions. An experimental investigation of this point is highly desirable and will be undertaken. The trend in r with temperature in table 1 may be due to the presence of eye modifiers not uniformly distributed in the population. The effects of these modifiers may be progressively obscured as temperature increases, through the more efficient operation of processes initiated by other genes which are more uniformly distributed. This explanation is, of course, purely formal but can be tested by investigating the parent-offspring and dorso-ventral correlations over a range of temperatures.

THEORETICAL

Hersh (1928) has investigated the inter-lobe correlation for a number of combinations of *B*, *BB*, and wild type over a range of temperatures. For comparison with my own results the data on *B* are most relevant. The correlation between lobes is moderately high at most temperatures and shows no systematic trend with temperature. The stock used in those experiments is the low selected forked Bar stock, obtained from Zeleny in 1921, and used by Hersh in other investigations (Hersh 1924 and 1927). No measure of genetic uniformity in the stock is available since no parent-offspring correlation is given. In view of the rigorous selection for low facet number in this stock, a high degree of uniformity may be assumed. However, a comparison of the facet temperature relation in 1924 with that in 1927 (compare table 3, Hersh 1927 with table 1, Hersh 1924) indicates that some germinal modification had taken place during the years following selection and inbreeding. This is indicated by the relatively higher count at all comparable temperatures in 1927. I have found in my own stocks a marked tendency toward accumulation of high modifiers following close inbreeding for many generations. This progressively accumulated genetic diversification is, perhaps, sufficient to account for the inter-lobe correlation.

Moreover, the use of a 3 to 4 day egg-laying period introduces an environmental variable which operates differentially on the flies in any one culture. Those flies developing from the early laid eggs are exposed to optimum conditions, whereas the flies from later eggs show effects of progressive depletion of food supply as evidenced by a decrease in facet number and general body size (LUCE 1931, MARGOLIS 1935). Data to be published elsewhere demonstrate clearly that poor culture conditions increase the inter-lobe correlations. PEARL (1906) observed a small but similar increase in size correlations in Chilomonas due to poor culture conditions. It is, of course, impossible to establish the degree to which the various factors mentioned contribute to the inter-lobe correlations observed, unless the genetic and environmental factors are controlled separately.

An interesting point of difference in HERSH'S 1928 data and my own, is in the relation of the facet ratio between lobes (D/V) to temperature. As illustrated in table 1 for stock A, D/V is constant for all temperatures, for both sexes. HERSH, on the other hand, finds that when log D is plotted against log V for each temperature a straight line is obtained. This, of course, means that $\frac{\log D}{\log V}$ is constant over the range of temperatures. From this fact HERSH concludes that the rate of formation of facets in the dorsal and ventral lobes is logarithmic in character. This analysis, however, tells us nothing concerning the rate of formation of facets in the individual under any specified set of conditions, but gives us the relation of facet number to temperature for dorsal and ventral lobes, respectively. This can be illustrated more clearly in the following manner. HERSH (1930) has shown that the facet-temperature relation for various combinations in the Bar series is exponential in character, conforming to the expression

$$y = ae^{rt}$$

where y is the number of facets, t the temperature, a and r constants, and e the base of the system of natural logarithms. This expression gives an excellent fit to the data. If now, both the dorsal and ventral lobes are exponential functions of temperature such that

$$D = ae^{rt} \qquad (1)$$

$$V = a^1e^{r^1t} \qquad (2)$$

then solving (1) and (2) in terms of t and eliminating t and e from the equations, D is related to V as a power function of the form

$$y = bx^k \qquad (3)$$

where $y = D$, $x = V$, and b and k can be evaluated in terms of the constants a, a^1, r, and r^1 in equations (1) and (2). This is, in fact, implicit in plotting log D against log V for different temperatures as carried out by HERSH. In plotting the data as indicated above it is temperature and not time which is implicit, so that no growth relation may be assumed.

Since, in my own data (table 1), it is $\frac{D}{V}$ rather than $\frac{\log D}{\log V}$ which is constant, some explanation for the difference is desirable. Considering again equations (1) and (2), in the special case where the constants r and r^1 are equal, the constant k in equation (3) which is equal to r/r^1 becomes equal to 1 so that the relation between D and V over the temperature range is rectilinear. It appears then that D and V in both HERSH's and my own data are related to temperature in the same manner but that HERSH's results conform to the more general relation of which my own represent a special case, for example when r and r^1 are equal.

In a further investigation of the facet relations in the dorsal and ventral lobes, HERSH (1931) has fitted the relative growth function to data on a series of Bar stocks differing from each other in respect to various mutant markers in different regions of the X chromosome. The experiment was conducted at 25° C. In applying the relative growth function to these data, HERSH points out that there is the assumption that increasing facet numbers in the two lobes represent progressive growth levels. Fifteen stocks were tested, some giving an excellent fit to the calculated curves, others a questionable fit, and still others rather wide departures. This statement is based merely on visual inspection of the curves rather than on any tests of goodness of fit.

The manner in which the facet numbers in dorsal and ventral lobes were calculated requires some consideration. In making these calculations for any one series, the facet data were seriated on the basis of total facet number instead of treating facet number in dorsal and ventral lobes as independent variates. The latter procedure is the one used in determining regression, and involves no assumptions concerning the relations of the variates. In his 1928 paper HERSH states that regression for those data was sensibly linear. If regression is likewise linear for the 1931 data, it is clear that the relative growth function cannot satisfactorily be fitted to data seriated with reference to either dorsal or ventral lobes treated as independent variates. It is of some interest, then, to determine the consequences of seriation of data on the basis of total eye size. Expressed in general terms the problem may be stated as follows: given two variables, D and V, such that $D+V=T$, assuming normal distributions for D and V, respectively, and 0 correlation, what will be the relation between D and V calculated from data seriated with reference to T?

This problem was attacked by building up a series of theoretically constructured correlation tables for D and V using the normal distribution, and assuming 0 correlation. Different relative magnitudes of the standard deviations and means were used for the two distributions. Analysis of these tables showed that for data seriated with reference to T there is a well defined relation between D and V despite the absence of any correlation. When the standard deviations for D and V differ, the relation is sigmoid; when the standard deviations are equal, the relation is rectilinear. The values of the means, however, do not affect the form of the relation.

It was of further interest to test the application of these conclusions to a set of data which approximated the conditions specified in the analysis, namely, normality of distribution of the variates and 0 correlation. For this purpose the data on facet number for the females of stock B, from experiments IIIa, IIIc, IIId, IIIe, IIIf, IIIh, and IIIi, were used (table 2). The data from the different experiments were then grouped together and biometric constants for dorsal and ventral lobes calculated. The values for the constants are as follows:

$$D = 30.10 \pm 0.237;\ \sigma_D = 3.484$$
$$V = 23.08 \pm 0.197;\ \sigma_V = 2.900$$
$$r = -0.0092 \pm 0.0680$$
$$N = 215$$

The data were then seriated with reference to total eye size and values of D and V calculated for each class. These values of D and V are plotted in figure 1, and are represented by open circles. The solid circles in figure 1 represent the values of D and V calculated from the same data, but seriated with reference to D. The latter represents graphically the regression of V on D calculated in the usual way. The curve in figure 1 represents a theoretially calculated curve using the means and standard deviations of dorsal and ventral lobes respectively and assuming normality of distribution of the variates as well as 0 correlation. The horizontal line represents the theoretical regression of V on D. The sigmoid character of the curve calculated from data seriated with respect to total facet number is clearly apparent. Were it not obvious that this is a consequence of the mathematical treatment of the data, one might be led to assume some functional relation between facet numbers in the two lobes. The absence of any such relation is, however, clearly indicated by the analysis of the data seriated with reference to either dorsal or ventral lobes, and by the absence of correlation between lobes. Considering the nature of the assumptions involved in the calculation of the theoretical curve, a remarkably good fit is obtained. The first point, for example, is based on only one individual. The whole question of the form of distribution of facet number in Bar will be treated elsewhere, although the present data indicate an excellent approximation to normality.

One must conclude, at least for the data at 28°, that facet number in Bar is independently determined in the two lobes. The factors which lead to the impression of some degree of interdependence as evidenced by correlation between lobes have already been discussed at some length but may be summarized briefly.

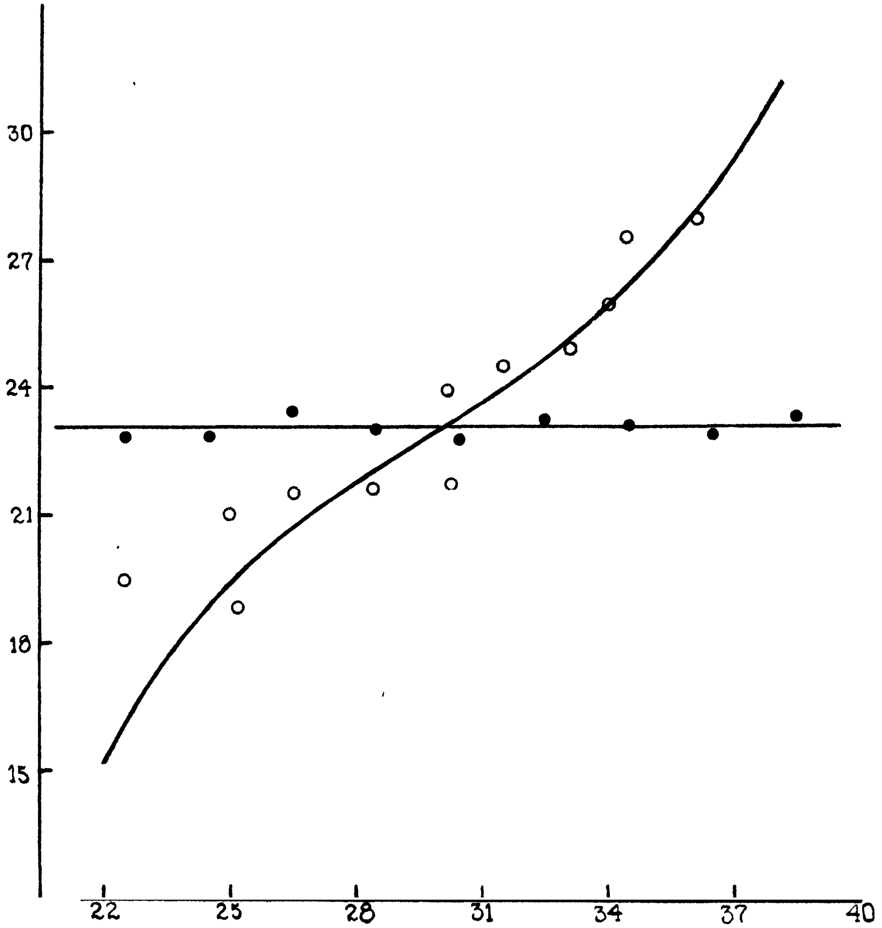

FIGURE 1. Ordinates—ventral facet number; abscissae—dorsal facet number. Solid circles represent data seriated with reference to facet number in dorsal lobe. Open circles represent data seriated with reference to total facet number. See text for calculation of curves.

1. Genetic heterogeneity in the population leads to a correlation between lobes since, although the lobes may vary independently of each other, the two lobes are covariant with respect to the genotype.

2. Environmental fluctuation operates in a similar manner. Facet num-

ber in either lobe varies with such factors as temperature, culture conditions, etc., so that variability in any one of these factors leads to concomitant variation in both lobes, in this way bringing about a correlation between lobes.

If this interpretation of the facet relations in the two lobes is correct, then we have a very simple method for testing the uniformity of both genetic and environmental factors in any experiment. Zero correlation should indicate uniformity of all factors affecting facet number. In this respect, the determination of inter-lobe correlations serves the same purpose as the left-right correlations for bristle number as used by PLUNKETT (1926) and subsequently by CHILD (1935). In the case of facet number, the left-right correlations are misleading since my data indicate some degree of developmental interdependence.

The question arises concerning the factors determining the lobing of the eye. DIETRICH (1907) has called attention to the fact that lobing is by no means uncommon among the Diptera. Moreover, this lobing is some times accompanied by differences in size of facets as well as histological pattern of the ommatidia in the two lobes. These facts are of interest since in Drosophila, superficially at least, there is no indication of lobing in the wild type eye, so that the Bar gene would seem to have introduced the lobing effect as a new feature. Recent unpublished observations of C. W. ROBERTSON of this laboratory, however, indicate that the ommatidia of the wild type eye are divisible histologically into two groups based upon the arrangement of the retinulae cells within each group. It appears then that the lobing in Bar is foreshadowed in the wild type, although the latter gives no superficial indication of this fact.

The apparent independence of facet number in the two lobes may be explained by at least two simple hypotheses. (1) The facet number in each lobe may be determined in a separate center. (2) Facet number may be determined as a whole, but an independent set of factors may operate to determine the lobing. If the factors determining the lobing operate independently of those determining facet number, then no correlation between lobes is to be expected. There appears at present to be no way of distinguishing definitely between these possibilities.

The observations and analysis of data on dorsal and ventral lobes of the eye in Bar-eyed Drosophila presented here show definitely that a number of factors may operate to produce an apparent relation between facet numbers in the two lobes where, in fact, no demonstrable functional relation exists. It is impossible to specify the manner in which each of these factors affects the end result, without experiments directed specifically toward that end. It is, however, clear that these factors should be evaluated in any analysis of the problem.

SUMMARY

Data on facet number in the dorsal and ventral lobes of the compound eye of Bar-eyed Drosophila are presented. Two unrelated but approximately genetically homogeneous stocks were used in the experiments. In stock A, the value of the correlation between lobes increases with decrease in temperature. This is interpreted as due to some residual genetic heterogeneity. In stock B, a series of experiments at 28°C indicate that the dorsoventral correlation is of the same magnitude as the parent-offspring correlation for the stock. It is concluded that genetic heterogeneity is adequate to account for the correlations observed. Variability in environmental agencies such as temperature and culture conditions also contribute to an increase in correlation between lobes. If these conclusions apply equally to all Bar stocks then the degree of correlation between lobes serves as a measure of uniformity of all factors, both genetic and environmental, which affect facet number.

An analysis on the effects of different methods of seriation of the data is presented. It is demonstrated that seriation of the facet data on the basis of total eye size leads to the impression of a functional relation between dorsal and ventral lobes where, in fact, no demonstrable relation exists, as evidenced by complete absence of regression or correlation.

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STUDIES ON HYBRID STERILITY IV. TRANSPLANTED TESTES IN DROSOPHILA PSEUDOOBSCURA

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INTERSPECIFIC hybrids may be equal or superior to their parents in somatic vigor and, at the same time, show disturbances in the gonads of such a nature that no functional gametes are produced. This antithesis between the general vigor on one hand and the degeneration of the reproductive tissues on the other constitutes an interesting developmental problem. In previous papers of this series (DOBZHANSKY 1934, 1936) it has been pointed out that there are two types of hybrid sterility, the "chromosomal" type in which meiosis is abnormal because of the structural non-correspondence of the two parental sets of chromosomes, and the "genic" type in which sterility is caused by interactions of complementary genes contributed by both parents. In the chromosomal type the phenomenon of sterility is an intracellular one. In the case of genic sterility, on the other hand, it is possible, *a priori*, that intercellular influences are involved. One might suppose, for example, that there are diffusible substances, not characteristic of the parental species, produced in the hybrid, and that such substances have specific effects on the reproductive system. The work of IRWIN and COLE (1936 a, b, c) on hybrids of doves and pigeons has in fact demonstrated, by means of immunogenetic reactions, the existence in the erythrocytes of such "hybrid" substances, that is, of specific substances present in the hybrid but absent in the parents. Of course, the hypothesis of the intercellular causation of sterility does not necessarily involve the assumption of specific hybrid substances. The fate of a gonad may be determined by the general metabolism (in the broad sense of the term) of the body containing it.

Transplantation of hybrid gonads to the parental forms, and *vice versa*, provides a direct method for testing the above hypothesis. By means of appropriate transplants, it is possible to determine whether hybrid sterility, in a particular case, is determined by the constitution of the gonad itself (autonomous development), or whether the genetic constitution of the surrounding tissues is a contributing factor (dependent development). Races A and B of *Drosophila pseudoobscura* provide favorable material for such studies. The F_1 males from interracial crosses are fully viable and vigorous, but show profound and characteristic disturbances in their spermatogenesis and are completely sterile. Except for the testes, the reproductive system appears to be quite normal.

MATERIAL AND METHODS

The technique of transplantation developed by EPHRUSSI and BEADLE (1935, 1936) for Drosophila has been used successfully by these workers for transplanting larval testes of *D. melanogaster* (unpublished). In case of *D. pseudoobscura* special thin-walled large-bore micro-pipettes were employed, but otherwise the technique was essentially the same as described for *D. melanogaster*. Larval testes must be handled with a good deal of care to avoid rupturing the delicate external membrane. Unless otherwise stated, the transplantations described below were made with larvae approaching the prepupal stage.

Four strains of *D. pseudoobscura* were used: wild type strains Zuni-5 (race A, strong), and Seattle-6 (race B, moderately weak), and mutant strains eosin magenta short (race A, rather strong), and scutellar dela (race B, intermediate). The two latter strains carry mutant genes affecting the eye color as well as that of the testicular envelope. The experiments were so arranged that it was possible to distinguish the implant from the testes of the host by color. Testis color in these experiments proved to develop autonomously, although non-autonomous development of this character has been observed in *D. simulans* (DOBZHANSKY 1931).

Eggs were collected over 24 hour periods at room temperature (about 22°C), then allowed to develop and hatch at 25°. Approximately 48 hours after collection, larvae were transferred to culture dishes and, from that time until transplantations were made, were grown at 19°. Larvae on which operations had been made were allowed to develop to maturity at 25°. In many cases, it was necessary to provide food after the operations; this was done by adding to the vial a small piece of standard food seeded with yeast.

The implanted testes, and in some cases those of the host, were fixed and stained in aceto-carmine, and smear preparations made. Spermatogenesis in normal and hybrid males has been described previously (DOBZHANSKY 1934). The difference in spermatogenesis between hybrid and normal males is characteristic enough, so that relatively slight modifications of either are readily detectable. In the hybrids the chromosomal pairing at meiosis is more or less suppressed, no second spermatocytes are formed, and the spermatids undergo a characteristically abnormal development. All of these abnormalities were looked for in preparations of the implanted testes and in the testes of the host, especial attention being paid to chromosome pairing at diakinesis and metaphase of the first meiotic division, since the process of pairing is known to be sensitive to external agents (BAKER 1936, BAUER, unpublished).

DEVELOPMENT OF IMPLANTED TESTES

Among 146 individuals in which testes were implanted in the larval stage, 22.8 per cent contained no implant when dissected, presumably because of loss or injury to the testis during the process of injection. Among those flies in which the implant had developed 61.1 per cent had the implant lying in the body cavity not connected with the reproductive organs of the host except by tracheal branches, 27.3 per cent had the implanted testis attached to one of the two vasa efferentia and one of the host's testes free in the body cavity, and in 11.5 per cent both the implant and one of the host's testes were attached to the same vas efferens (fig. 1).

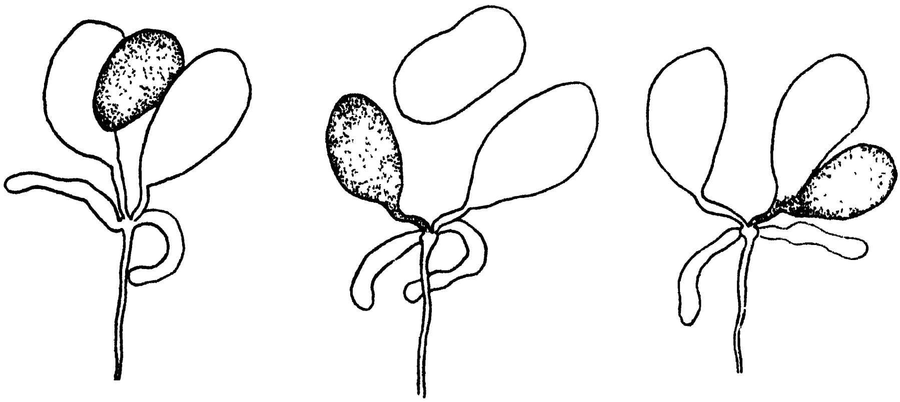

FIGURE 1.—The internal reproductive organs of eosin magenta males with A ♀ × B ♂ hybrid testes implanted in them. The implanted testes are in this case red (stippled in the figure), and those of the host pale yellow (white in the figure). Left—both host's testes attached to the vasa efferentia, the implanted testis free. Middle—the implant attached, one of the host's testes free. Right—implanted and host testes attached to the same vas efferens.

The connection established between the implanted testis and the vas efferens of the host may become functional, as shown by the fact that the sperm from the implant may be used in fertilization (see below). When two testes are attached to the same vas efferens it is not known whether the connection is functional for both of them. The data indicate that race A testes implanted in race B hosts become attached to the ducts of the host more frequently than race B testes in race A hosts; however the numbers of individuals are so small that this conclusion cannot be established with certainty. In the hybrids in which the testes of the host are small and the implanted testis of one of the pure races is much larger, no tendency is observed for the implant to be attached in preference to the host's testes.

In a control experiment testes of eosin magenta short (race A) were implanted in Zuni-5 (race A) larvae. Spermatogenesis in the implanted testes was found to be normal, showing that the process of transplantation as such does not affect the normal development of the testis. During the entire study preparations of testes found lying free in the body cavity were kept separate from those of testes attached to the ducts; no difference was found in spermatogenesis in these two classes. Furthermore, in some cases testes were implanted into female hosts. A testis which develops in a female body is somewhat smaller in size than one which develops in a male. A similar observation was made in the case of certain types of gynandromorphs in *D. simulans* (DOBZHANSKY 1931). In case of attachment to a female duct no study of spermatogenesis was made, but no abnormalities in spermatogenesis were detected where the testis was not attached to an oviduct.

TRANSPLANTS BETWEEN RACES

Nine Zuni (race A) males with dela (B) testis implants, and seven eosin magenta (A) males with Seattle (B) implants were studied cytologically. Spermatogenesis was found to be quite normal in all stages. In 215 first spermatocytes, the normal number of bivalents, namely four, was observed. *D. pseudoobscura* has five pairs of chromosomes, but the fifth pair is so small that the bivalent formed by it is not usually seen at the meiotic stages. In one cell the X and Y chromsomes were unpaired, but such failure of pairing is occasionally observed in normal testes (DARLINGTON 1934). In the testes of the host spermatogenesis was observed to be normal. In 85 first spermatocytes, four bivalents were observed in each, and in one cell there were three bivalents and two univalents (X and Y chromosomes). One cyst of tetraploid spermatocytes was found. Six dela (B) males with Zuni (A) implanted testes also showed normal spermatogenesis in both the host and the implant. Four bivalents were seen in 99 first spermatocytes in the implanted testes and in 29 spermatocytes in the host.

In order to demonstrate that spermatogenesis is not only visibly normal but also leads to the production of functional sperm, eosin magenta males with Seattle implants were crossed to eosin magenta females. Nine out of ten such crosses produced offspring, which in six cases consisted of eosin magenta individuals only, and in three cases of eosin magenta as well as wild type individuals. Clearly, in the six former only the testes of the host, and in the three latter both the host's and the implanted testes functioned. Dela males with Zuni testes implanted were crossed to dela females; one out of four cultures produced only dela flies, and the remaining three both dela and wild type flies. The cases where no offspring were produced from the implanted testes are presumably those in which the implanted testis did not become attached to the vasa efferentia. The conclusion is justified that a race A testis developing in a race B body neither loses its ability to function nor produces any disturbance in the testes of the host. The same is true for race B testes implanted in race A.

IMPLANTS FROM PURE RACES TO HYBRIDS

Four experiments of this type were made: (1) eosin magenta A testes were implanted into Zuni A ♀ × Seattle B ♂ hybrids, (2) dela B into Zuni A ♀ × Seattle B ♂, (3) eosin magenta A into Seattle B ♀ × Zuni A ♂, and (4) dela B into Seattle B ♀ × Zuni A ♂. In race B ♀ × race A ♂ hybrid males, the testes are visibly smaller than in either pure race or in A ♀ × B ♂ hybrids. Therefore, in the third and fourth experiments the implanted testes proved to be much larger than those of the hosts, while in the first and second experiments the implants were of the same or of slightly smaller size than those of the hosts. The implanted testes were studied cytologically and all stages of spermatogenesis proved to be normal. In the first experiment ten testes were examined and 54 first spermatocytes with four bivalents in each were seen; in the second experiment eight testes were examined and 68 spermatocytes with four and 1 with three bivalents (X and Y unpaired) found; in the third experiment five testes were examined and 76 cells with four bivalents found; in the fourth experiment eight testes were examined and 49 cells with four and 2 cells with three bivalents seen (X and Y chromosomes unpaired in one cell and an autosome unpaired in another).

About 12 males from each experiment were crossed to females homozygous for the recessive genes carried by the implanted testes. Since the hybrid males are normally sterile, the production of offspring from these matings would mean either that the implanted testes are able to function or that the presence of the implant induces fertility in the testes of the host. The presence of mutant genes as markers enables one to discriminate between these possibilities. Two males from each of the first, third and fourth experiments proved to be fertile, and the offspring showed that the sperm must have come from the implanted testes. We can conclude: (1) that the functioning of the testes of the pure races is not interfered with when they develop in the body of a hybrid, (2) that the testes of a hybrid host do not become fertile in the presence of a normally developed implant, and (3) that the ducts of the reproductive system in the hybrid males are normal and potentially functional.

IMPLANTS FROM HYBRIDS TO PURE RACES

Four experiments were made in which testes from hybrid males were implanted in males of pure races: testes of Seattle B ♀ × Zuni A ♂ hybrids in (1) eosin magenta A males and (2) dela B males, and testes of Zuni A ♀ × Seattle B ♂ hybrids in (3) eosin magenta and (4) dela males. In all cases autonomous development of the implant was observed. The B ♀ ×A ♂ hybrid testes were small, and those of the A ♀ ×B ♂ hybrids were about equal in size to testes of the pure race hosts. In the first, second and fourth experiments seven implants were studied cytologically; in the third experiment three testes were studied. Spermatogenesis was found to be similar to that normally observed in hybrid testes; disturbance or complete lack of meiotic pairing, absence of second spermatocytes, degeneration of the spermatids, and other characteristic abnormalities. Four testes of the hosts were also examined, and found to contain normal stages of spermatogenesis. Five males from the first experiment were tested for fertility, and all proved to be fertile. It follows that the presence of a hybrid testis in the body of a normal male induces no change either in the implant or in the host.

TRANSPLANTATION AT EARLIER STAGES

In the experiments described above, both the donors and the hosts were nearly mature larvae (since the results were uniform, data regarding the precise ages of larvae in each individual experiment have been omitted). We have seen that under these conditions the development of the implanted as well as of the host testes is autonomous. It follows that in the mature larvae the fate of the testis is already determined, in the sense that its transplantation into the body of a foreign race does not produce any effect on spermatogenesis. The possibility remains however that if the transplantations were made at an earlier stage the results would be different. As an analogy, the findings of BAKER (1935) may be mentioned here. BAKER has shown that there is a sensitive period for the influence of high temperature on the testes of *D. pseudoobscura*, and that this sensitive period lies in the prepupal stage, that is, precisely the stage at which the majority of our transplantation experiments were made.

In the following two experiments the donor and the host larvae were much younger than in those experiments reported above, namely three days before pupation, which corresponds to the end of the second or the beginning of the third larval instars. After the operations were made, the larvae were given food sufficient to cover the period to pupation. The experiments were: (1) implants of Zuni A and (2) of Seattle B testes in dela B ♀ ×Zuni A ♂ hybrid larvae. Cytological examinations were made on five testes from the first experiment and on seven testes from the second. All stages of spermatogenesis were found to be normal: 72 spermatocytes in the first and 64 in the second experiment were found to contain four bivalents each; one testis from the first experiment contained a cyst of tetraploid cells, and in another testis a spermatocyte with only three bivalents (X and Y unpaired) was found.

About a dozen males from each of the two experiments were tested for fertility by crossing them to eosin magenta and to dela females respectively. Three males from the first, and none from the second experiment proved fertile. This low frequency of fertile males is due to the fact (ascertained by dissection) that in the large majority of males the implanted testis was not attached to the vasa efferentia.

DISCUSSION

Only few transplantation studies have been made which bear on the question of hybrid sterility in other insects. BYTINSKI-SALZ (1933) transplanted the ovaries of the pupae of the hybrid *Celerio euphorbiae* ♀ × *C. gallii* ♂ into pupae of the parental species. Normally the ovaries of the donor do not develop to maturity, since the hybrid does not survive the pupal stage; but the transplants were observed to grow and to form nearly mature eggs. It remains unclear whether in these experiments a transformation of a sterile gonad into a fertile one is involved, or (which seems vastly more probable) merely a survival of an organ which would normally die together with the organism containing it. EPHRUSSI and BEADLE (1935) transplanted the ovaries of *Drosophila simulans* to *D. melanogaster*, and found that the implant produces functional ova (the hybrid between these two species is completely sterile). Thus, the development of the ovary of one species in the body of another does not lead to a condition resembling hybridity. EPHRUSSI and MONOD (unpublished) transplanted the ovaries of *D. melanogaster* into *melanogaster* × *simulans* hybrid larvae. The resulting adult flies have three ovaries; two ovaries of the host are rudimentary (as they normally are in the hybrid), while in the implanted ovary apparently normal ova are present. The conclusion is that the ovary of *melanogaster* is not affected by development in the hybrid organism.

The data presented in the present paper are consistent throughout. The testes of one pure race develop normally in the body of the other race and in the hybrids, and the hybrid testes develop autonomously in the body of either race. Cytological investigation shows that there is no detectable influence of the host on the implant. Pure race testes produce functional sperm in the hybrid body, and the hybrid testes never develop fertility when implanted into the pure races. The development of the testes is autonomous, "herkunftsgemäss" and not "ortsgemäss." It follows that the condition of the gonad is determined by the genetic structure of the cells within the gonad and not by interactions between the gonad and influences emanating from other parts of the body. The only escape from this conclusion is to suppose that in our experiments the transplantations were made after the period during which the gonad may be sensitive to the influences of other parts of the body has passed. Such a supposition has little chance to be correct, not only because some of the experiments were made in rather young larvae, but also because in *D. pseudoobscura* the proliferation of the primary spermatogonia continues during the whole of the pupal and large part of the adult life. At the stage at which the transplants were made the gonads contain mainly spermatogonia and a few spermatocytes. In other species of Drosophila, notably in *melanogaster* and *simulans*, the situation is different in this respect.

The phenomenon of sterility in the interracial hybrids in *D. pseudoobscura* must be intragonadal in nature. It would seem that only those external influences can modify the behavior of the sex cells which can act directly on the gonads. No "somatic induction" is likely to occur. This consideration is of importance for planning experiments which have to do with the physiology of sterility. It may be noted here that in cases where the sterility is of the chromosomal type (DOBZHANSKY 1934, 1936) the phenomenon is almost of necessity an intracellular one; but where genic sterility is concerned both intra- and intercellular causations are *a priori* equally possible. Hence, the results of this investigation of *D. pseudoobscura* cannot be generalized further. It should also be noted that transplantation of gonads between species may be expected on *a priori* grounds to produce in some cases a suppression of the implants. Such a suppression may not mean however that a condition resembling hybrid sterility is induced, unless it is demonstrated that cytological abnormalities resembling those found in sterile hybrids are apparent.

SUMMARY

Larval testes of race A were implanted into race B larvae, and *vice versa*. Testes of A×B hybrids were implanted into the pure races, and pure race testes were implanted into hybrids. Study of spermatogenesis has shown that the development of the implanted testes as well as of those of the host is autonomous in all cases. It follows that in *Drosophila pseudoobscura* the structure of the gonad is determined by its own genetic constitution and not by the genetic constitution of the surrounding soma.

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